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(54) Title: NOVEL OSTEOINDUCTIVE COMPOSITIONS

(57) Abstract

Human and bovine bone inductive factor products and processes. The factors may be produced by recombinant techniques and are useful in the research and treatment of bone and periodontal defects.

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NOVEL OSTEOINDUCTIVE COMPOSITIONS

The present invention relates to novel proteins and processes for obtaining them. These proteins are capable of inducing cartilage and bone formation.

Background

Bone is a highly specialized tissue characterized by an extensive matrix structure formed of fibrous bundles of the protein collagen, and proteoglycans, noncollagenous proteins, lipids and acidic proteins. The processes of bone formation and renewal/repair of bone tissue, which occur continuously throughout life, are performed by specialized cells. Normal embryonic long bone development is preceded by formation of a cartilage model. Bone growth is presumably mediated by "osteoblasts" (bone-forming cells), while remodeling of bone is apparently accomplished by the joint activities of bone-resorbing cells, called "osteoclasts" and osteoblasts. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

Brief Description of the Invention

The present invention provides novel proteins in purified form. Specifically, four of the novel proteins are designated BMP-1, BMP-2 Class I (or BMP-2), BMP-3, and BMP-2 Class II (or BMP-4) wherein BMP is bone morphogenic protein. These proteins are characterized by peptide sequences the same as or substantially homologous to amino acid sequences illustrated in Tables II through VIII below. They are capable of inducing bone formation at a predetermined site. These bone inductive factors are further characterized by biochemical and biological characteristics including activity at a concentration of 10 to 1000ng/gram of bone in an *in vivo* rat bone formation assay described below. Proteins of this invention may be encoded by the DNA sequences depicted in the Tables or by sequences capable

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of hybridizing thereto and coding for polypeptides with bone growth factor biological properties or other variously modified sequences demonstrating such properties.

One of the proteins of the invention is designated BMP-1. A portion of the human BMP-1 or hBMP-1 is characterized by the same or substantially the same peptide sequence as that of amino acid #1 through amino acid #37 of Table V, below which represents a genomic hBMP-1 fragment or amino acid #1 through amino acid #730 of Table VI which represents the hBMP-1 cDNA. hBMP-1 or a related bone inductive factor may be further characterized by at least a portion of these sequences. These peptide sequences are encoded by the same or substantially the same DNA sequence, as depicted in nucleotide #3440 through nucleotide #3550 of Table V and in nucleotide #36 through nucleotide #2225 of Table VI, respectively. These hBMP-1 polypeptides are further characterized by the ability to induce bone formation. hBMP-1 demonstrates activity in an _in vivo_ rat bone formation assay at a concentration of 10 to 1000ng/gram of bone.

The homologous bovine growth factor of the invention, designated bBMP-1, is characterized by a peptide sequence containing the same or substantially the same sequence as that of amino acid #1 through amino acid #37 of Table II below which represents a genomic bBMP-1 fragment. This peptide sequence is encoded by the same or substantially the same DNA sequence as depicted in nucleotide #294 through nucleotide #404 of Table II. The bovine peptide sequence identified in Table II below is also 37 amino acids in length. bBMP-1 is further characterized by the ability to induce bone formation.

Another bone inductive protein composition of the invention is designated BMP-2 Class I (or BMP-2). It is characterized by at least a portion of a peptide sequence the same or substantially the same as that of amino acid #1 through amino acid #396 of Table VII which represents the cDNA hBMP-2 Class I. This peptide sequence is encoded by the same or

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substantially the same DNA sequence, as depicted in nucleotide #356 through nucleotide #1543 of Table VII. The human peptide sequence identified in Table VII is 396 amino acids in length. hBMP-2 or related bone inductive proteins may also be characterized by at least a portion of this peptide sequence. hBMP-2 Class I is further characterized by the ability to induce bone formation.

The homologous bovine bone inductive protein of the invention designated bBMP-2 Class I (or bBMP-2), has a DNA sequence identified in Table III below which represents the genomic sequence. This bovine DNA sequence has a prospective 129 amino acid! coding sequence followed by approximately 205 nucleotides (a presumptive 3' non-coding sequence). bBMP-2, Class I is further characterized by the ability to induce bone formation. A further bone inductive protein composition of the invention is designated BMP-2 Class II or BMP-4. The human protein hBMP-2 Class II (or hBMP-4) is characterized by at least a portion of the same or substantially the same peptide sequence between amino acid #1 through amino acid #408 of Table VIII, which represents the cDNA of hBMP-2 Class II. This peptide sequence is encoded by at least a portion of the same or substantially the same DNA sequence as depicted in nucleotide #403 through nucleotide #1626 of Table VIII. This factor is further characterized by the ability to induce bone formation.

Still another bone inductive factor of the invention, BMP-3, is represented by the bovine homolog bBMP-3. bBMP-3 is characterized by the DNA sequence and amino acid sequence of Table IV A and B which represents the bovine genomic sequence. It is characterized by at least a portion of a peptide sequence the same or substantially the same as amino acid #1 through amino acid #175 of Table IV A and B. BMP-3 is further characterized by the ability to induce bone formation. The bovine factor may be employed as a tool for obtaining the analogous human BMP-3 protein or other mammalian bone inductive proteins. The proper characterization of this bovine bone

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inductive factor provides the essential "starting point" for the method employing this sequence. The method, employing techniques known to those skilled in the art of genetic engineering, involves using the bovine DNA sequence as a probe to screen a human genomic or cDNA library; and identifying the DNA sequences which hybridize to the probes. A clone with a hybridizable sequence is plaque purified and the DNA isolated therefrom, subcloned and subjected to DNA sequence analysis. Thus as another aspect of this invention is a human protein hBMP-3, produced by this method.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of one or more bone growth factor polypeptides according to the invention in a pharmaceutically acceptable vehicle. These compositions may further include other therapeutically useful agents. They may also include an appropriate matrix for delivering the proteins to the site of the bone defect and for providing a structure for bone growth. These compositions may be employed in methods for treating a number of bone defects and periodontal disease. These methods, according to the invention, entail administering to a patient needing such bone formation an effective amount of at least one of the novel proteins BMP-1, BMP-2 Class I, BMP-2 Class-II, and BMP-3 as described herein.

Still a further aspect of the invention are DNA sequences coding on expression for a human or bovine polypeptide having the ability to induce bone formation. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in Tables II through VIII. Alternatively, a DNA sequence which hybridizes under stringent conditions with the DNA sequences of Tables II - VIII or a DNA sequence which hybridizes under non-stringent conditions with the illustrated DNA sequences and which codes on expression for a protein having at least one bone growth factor biological property are included in the present invention. Finally, allelic or other variations of the

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sequences of Tables II through VIII, whether such nucleotide
changes result in changes in the peptide sequence or not, are
also included in the present invention.

Still a further aspect of the invention is a vector
containing a DNA sequence as described above in operative
association with an expression control sequence. Such vector
may be employed in a novel process for producing a bone
growth factor polypeptide in which a cell line transformed
with a DNA sequence encoding expression of a bone growth
factor polypeptide in operative association with an expression
control sequence therefor, is cultured. This claimed process
may employ a number of known cells as host cells for expression
of the polypeptide. Presently preferred cell lines are
mammalian cell lines and bacterial cells.

Other aspects and advantages of the present invention will
be apparent upon consideration of the following detailed
description and preferred embodiments thereof.

Detailed Description of the Invention

The proteins of the present invention are characterized
by amino acid sequences or portions thereof the same as or
substantially homologous to the sequences shown in Tables II
- VIII below. These proteins are also characterized by the
ability to induce bone formation.

The bone growth factors provided herein also include
factors encoded by the sequences similar to those of Tables
II - VIII, but into which modifications are naturally provided
(e.g. allelic variations in the nucleotide sequence which may
result in amino acid changes in the polypeptide) or deliberately
engineered. For example, synthetic polypeptides may wholly or
partially duplicate continuous sequences of the amino acid
residues of Tables II - VIII. These sequences, by virtue of
sharing primary, secondary, or tertiary structural and
conformational characteristics with bone growth factor
polypeptides of Tables II - VIII may possess bone growth factor
biological properties in common therewith. Thus, they may be

employed as biologically active substitutes for naturally-occurring bone growth factor polypeptides in therapeutic processes.

Other specific mutations of the sequences of the bone growth factors described herein involve modifications of one or both of the glycosylation sites. The absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at one or both of the asparagine-linked glycosylation recognition sites present in the sequences of the bone growth factors shown in Tables II-VIII. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for bone growth factors. These DNA sequences include those depicted in Tables II - VIII in a 5' to 3' direction and those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences of Tables II - VIII.

DNA sequences which hybridize to the sequences of Tables II - VIII under relaxed hybridization conditions and which code on expression for bone growth factors having bone growth factor biological properties also encode bone growth factors of the invention. For example, a DNA sequence which shares regions of significant homology, e.g., sites of glycosylation

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or disulfide linkages, with the sequences of Tables II - VIII
and encodes a bone growth factor having one or more bone
growth factor biological properties clearly encodes a member
of this novel family of growth factors, even if such a DNA
sequence would not stringently hybridize to the sequence of
Tables II - VIII.

Similarly, DNA sequences which code for bone growth factor
polypeptides coded for by the sequences of Tables II - VIII,
but which differ in codon sequence due to the degeneracies of
the genetic code or allelic variations (naturally-occurring base
changes in the species population which may or may not result
in an amino acid change) also encode the novel growth factors
described herein. Variations in the DNA sequences of Tables
II -VIII which are caused by point mutations or by induced
modifications to enhance the activity, half-life or production
of the polypeptides encoded thereby are also encompassed in the
invention.

Another aspect of the present invention provides a novel
method for producing the novel osteoinductive factors. The
method of the present invention involves culturing a suitable
cell or cell line, which has been transformed with a DNA
sequence coding on expression for a novel bone growth factor
polypeptide of the invention, under the control of known
regulatory sequences. Suitable cells or cell lines may be
mammalian cells, such as Chinese hamster ovary cells (CHO).
The selection of suitable mammalian host cells and methods for
transformation, culture, amplification, screening and product
production and purification are known in the art. See, e.g.,
Gething and Sambrook, *Nature*, 293:620-625 (1981), or
alternatively, Kaufman et al, *Mol. Cell. Biol.*, 5(7):1750-1759
(1985) or Howley et al, U.S. Patent 4,419,446. Another suitable
mammalian cell line, which is described in the accompanying
examples, is the monkey COS-1 cell line. A similarly useful
mammalian cell line is the CV-1 cell line.

Bacterial cells are suitable hosts. For example, the

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various strains of E. coli (e.g., HB101, MC1061) are well-known
as host cells in the field of biotechnology. Various strains
of B. subtilis, Pseudomonas, other bacilli and the like may also
be employed in this method.

Many strains of yeast cells known to those skilled in the
art are also available as host cells for expression of the
polypeptides of the present invention. Additionally, where
desired, insect cells may be utilized as host cells in the
method of the present invention. See, e.g. Miller et al,
Genetic Engineering, 8:277-298 (Plenum Press 1986) and
references cited therein.

Another aspect of the present invention provides vectors
for use in the method of expression of these novel
osteoinductive polypeptides. Preferably the vectors contain
the full novel DNA sequences described above which code for
the novel factors of the invention. Additionally the vectors
also contain appropriate expression control sequences permitting
expression of the bone inductive protein sequences.
Alternatively, vectors incorporating modified sequences as
described above are also embodiments of the present invention
and useful in the production of the bone inductive proteins.
The vectors may be employed in the method of transforming
cell lines and contain selected regulatory sequences in
operative association with the DNA coding sequences of the
invention which are capable of directing the replication and
expression thereof in selected host cells. Useful regulatory
sequences for such vectors are known to one of skill in the
art and may be selected depending upon the selected host
cells. Such selection is routine and does not form part of
the present invention.

A protein of the present invention, which induces bone
growth in circumstances where bone is not normally formed, has
application in the healing of bone fractures. An osteogenic
preparation employing one or more of the proteins of the
invention may have prophylactic use in closed as well as open

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fracture reduction and also in the improved fixation of
artificial joints. De novo bone formation induced by an
osteogenic agent contributes to the repair of congenital,
trauma induced, or oncologic resection induced craniofacial
defects, and also is useful in cosmetic plastic surgery. An
osteogenic factor of the invention may be valuable in the
treatment of periodontal disease, and in other tooth repair
processes. Such agents may provide an environment to attract
bone-forming cells, stimulate growth of bone-forming cells or
induce differentiation of progenitors of bone-forming cells.
Of course, the proteins of the invention may have other
therapeutic uses.

A further aspect of the invention is a therapeutic method
and composition for repairing fractures and other conditions
related to bone defects or periodontal diseases. Such a
composition comprises a therapeutically effective amount of at
least one of the bone inductive factor proteins of the
invention. The bone inductive factors according to the
present invention may be present in a therapeutic composition
in admixture with a pharmaceutically acceptable vehicle or
matrix. Further therapeutic methods and compositions of the
invention comprise a therapeutic amount of a bone inductive
factor of the invention with a therapeutic amount of at least
one of the other bone inductive factors of the invention.
Additionally, the proteins according to the present invention
or a combination of the proteins of the present invention may
be co-administered with one or more different osteoinductive
factors with which it may interact. Further, the bone inductive
proteins may be combined with other agents beneficial to the
treatment of the bone defect in question. Such agents include,
but are not limited to various growth factors. The preparation
of such physiologically acceptable protein compositions,
having due regard to pH, isotonicity, stability and the like,
is within the skill of the art.

In particular, BMP-1 may be used individually in a

composition. BMP-1 may also be used   in combination with
one or more of the other proteins of the invention. BMP-1
and BMP-2 Class I may be used in combination. BMP-1 and BMP-
2 Class II may also be used in combination. BMP-1 and BMP-3
may be used in combination. Furthermore, BMP-1 may be used
in combination with two or three of the other proteins of the
invention. For example, BMP-1, BMP-2 Class I, and BMP-2
Class II may be combined. BMP-1 may also be combined with
BMP-2 Class I, and BMP-3. Further, BMP-1 may be combined
with BMP-2 Class II, and BMP-3. BMP-1, BMP-2 Class I, BMP-2
Class II, and BMP-3 may be combined.

BMP-2 Class I may be used individually in a pharmaceutical
composition. BMP-2 Class I may also be used in combination
with one or more of the other proteins of the invention.
BMP-2 Class I may be combined with BMP-2 Class II. It may
also be combined with BMP-3. Further BMP-2 Class I may be
combined with BMP-2 Class II and BMP-3.

BMP-2 Class II may be used individually in pharmaceutical
composition. In addition, it may be used in combination with
other proteins as identified above. Further it may be used in
combination with BMP-3.

BMP-3 may be used individually in a composition. It may
further be used in the various combinations identified above.

The therapeutic method includes locally administering the
composition as an implant or device. When administered, the
therapeutic composition for use in this invention is, of course,
in a pyrogen-free, physiologically acceptable form. Further,
the composition may desirably be encapsulated or injected in
a viscous form for delivery to the site of bone damage.
Preferably, the bone growth inductive factor composition
would include a matrix capable of delivering the bone inductive
factor to the site of bone damage, providing a structure for
the developing bone and cartilage and optimally capable of
being resorbed into the body. Such matrices may be formed of
other materials presently in use for other implanted medical

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applications.

The choice of material is based on, for example, biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. Similarly, the application of the osteoinductive factors will define the appropriate formulation. Potential matrices for the osteoinductive factors may be biodegradable and chemically defined, such as, but not limited to calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyanhydrides; biodegradable and biologically well defined, such as bone or dermal collagen, other pure proteins or extracellular matrix components; nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics; or combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics might also be altered in composition, such as in calcium-aluminate-phosphate and processing to alter for example, pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of such a growth factor, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the composition of BMP's. The addition of other known growth factors, such as IGF 1 (insulin like growth factor 1), to the final composition, may also effect the dosage. Generally, the dosage regimen should be in the range of approximately 10 to 10^6 nanograms of protein per gram of bone weight desired. Progress can be monitored by periodic assessment of bone growth and/or repair, e.g. x-rays. Such therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity

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in bon inductive factors. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the bone inductive factors of the present invention.

The following examples illustrate practice of the present invention in recovering and characterizing the bovine proteins and employing them to recover the human proteins, obtaining the human proteins and in expressing the proteins via recombinant techniques.

EXAMPLE I

Isolation of Bovine Bone Inductive Factor

Ground bovine bone powder (20-120 mesh, Helitrex) is prepared according to the procedures of M. R. Urist et al., Proc. Natl Acad. Sci USA, 70:3511 (1973) with elimination of some extraction steps as identified below. Ten kgs of the ground powder is demineralized in successive changes of 0.6N HCl at 4°C over a 48 hour period with vigorous stirring. The resulting suspension is extracted for 16 hours at 4°C with 50 liters of 2M $CaCl_2$ and 10mM ethylenediamine-tetraacetic acid [EDTA], and followed by extraction for 4 hours in 50 liters of 0.5M EDTA. The residue is washed three times with distilled water before its resuspension in 20 liters of 4M guanidine hydrochloride [GuCl], 20mM Tris (pH 7.4), 1mM N-ethylmaleimide, 1mM iodoacetamide, 1mM phenylmethylsulfonyl fluorine as described in Clin. Orthop. Rel. Res., 171: 213 (1982). After 16 to 20 hours the supernatant is removed and replaced with another 10 liters of GuCl buffer. The residue is extracted for another 24 hours.

The crude GuCl extracts are combined, concentrated approximately 20 times on a Pellicon apparatus with a 10,000 molecular weight cut-off membrane, and then dialyzed in 50mM Tris, 0.1M NaCl, 6M urea (pH7.2), the starting buffer for the first column. After extensive dialysis the protein is loaded on a 4 liter DEAE cellulose column and the unbound fractions

are coll cted.

Th unbound fractions are concentrated and dialyzed against
50mM NaAc, 50mM NaCl (pH 4.6) in 6M urea. The unbound fractions
are applied to a carboxymethyl cellulose column. Protein not
bound to the column is removed by extensive washing with
starting buffer, and the bone inductive factor containing
material desorbed from the column by 50mM NaAc, 0.25mM NaCl,
6M urea (pH 4.6). The protein from this step elution is con-
centrated 20- to 40- fold, then diluted 5 times with 80mM
KPO_4, 6M urea (pH6.0). The pH of the solution is adjusted to
6.0 with 500mM K_2HPO_4. The sample is applied to an
hydroxylapatite column (LKB) equilibrated in 80mM KPO_4, 6M
urea (pH6.0) and all unbound protein is removed by washing
the column with the same buffer. Bone inductive factor
activity is eluted with 100mM KPO_4 (pH7.4) and 6M urea.

The protein is concentrated approximately 10 times, and
solid NaCl added to a final concentration of 0.15M.   This
material is applied to a heparin - Sepharose column equilibrated
in 50mM KPO_4, 150mM NaCl, 6M urea (pH7.4). After extensive
washing of the column with starting buffer, a protein with bone
inductive factor activity is eluted by 50mM KPO_4, 700mM NaCl,
6M urea (pH7.4). This fraction is concentrated to a minimum
volume, and 0.4ml aliquots are applied to Superose 6 and
Superose 12 columns connected in series, equilibrated with 4M
GuCl, 20mM Tris (pH7.2) and the columns developed at a flow
rate of 0.25ml/min. The protein demonstrating bone inductive
factor activity has a relative migration corresponding to
approximately 30,000 dalton protein.

The above fractions are pooled, dialyzed against 50mM NaAc,
6M urea (pH4.6), and applied to a Pharmacia MonoS HR column.
The column is developed with a gradient to 1.0M NaCl, 50mM
NaAc, 6M urea (pH4.6). Active fractions are pooled and brought
to pH3.0 with 10% trifluoroacetic acid (TFA). The material is
applied to a 0.46 x 25cm Vydac C4 column in 0.1% TFA and the
column developed with a gradient to 90% acetonitrile, 0.1% TFA

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(31.5% ac tonitrile, 0.1% TFA to 49.5% acetonitrile, 0.1% TFA
in 60 minutes at 1ml per minute). Active material is eluted
at approximately 40-44% acetonitrile. Aliquots of the appro-
priate fractions are iodinated by one of the following methods:
P. J. McConahey et al, _Int. Arch. Allergy_, 29:185-189 (1966);
A. E. Bolton et al, _Biochem J._, 133:529 (1973); and D. F.
Bowen-Pope, _J. Biol. Chem._, 237:5161 (1982). The iodinated
proteins present in these fractions are analyzed by SDS gel
electrophoresis and urea Triton X 100 isoelectric focusing.
At this stage, the bone inductive factor is estimated to be
approximately 10-50% pure.

EXAMPLE II

Characterization of Bovine Bone Inductive Factor

A. Molecular Weight

Approximately 20ug protein from Example I is lyophilized
and redissolved in 1X SDS sample buffer. After 15 minutes of
heating at 37°C, the sample is applied to a 15% SDS
polyacrylamide gel and then electrophoresed with cooling. The
molecular weight is determined relative to prestained molecular
weight standards (Bethesda Research Labs). Immediately after
completion, the gel lane containing bone inductive factor is
sliced into 0.3cm pieces. Each piece is mashed and 1.4ml of
0.1% SDS is added. The samples are shaken gently overnight at
room temperature to elute the protein. Each gel slice is
desalted to prevent interference in the biological assay. The
supernatant from each sample is acidified to pH 3.0 with 10%
TFA, filtered through a 0.45 micron membrane and loaded on a
0.46cm x 5cm C4 Vydac column developed with a gradient of 0.1%
TFA to 0.1% TFA, 90% CH_3CN. The appropriate bone inductive
factor - containing fractions are pooled and reconstituted with
20mg rat matrix. In this gel system, the majority of bone
inductive factor fractions have the mobility of a protein
having a molecular weight of approximately 28,000 - 30,000
daltons.

B.   Isoelectric Focusing

     The isoelectric point of bone inductive factor activity
is determined in a denaturing isoelectric focusing system. The
Triton X100 urea gel system (Hoeffer Scientific) is modified
as follows: 1) 40% of the ampholytes used are Servalyte
3/10; 60% are Servalyte 7-9.   2) The catholyte used is 40mM
NaOH.   Approximately 20ug of protein from Example I is
lyophilized, dissolved in sample buffer and applied to the
isoelectrofocusing gel. The gel is run at 20 watts, 10°C for
approximately 3 hours. At completion the lane containing
bone inductive factor is sliced into 0.5 cm slices. Each
piece is mashed in 1.0ml 6M urea, 5mM Tris (pH 7.8) and the
samples agitated at room temperature. The samples are
acidified, filtered, desalted and assayed as described above.
The major portion of activity as determined in the assay
described in Example III migrates in a manner consistent with
a pI of 8.8 - 9.2.

C.   Subunit Characterization

     The subunit composition of bone inductive factor is also
determined. Pure bone inductive factor is isolated from a
preparative 15% SDS gel as described above. A portion of the
sample is then reduced with 5mM DTT in sample buffer and
re-electrophoresed on a 15% SDS gel. The approximately 30kd
protein yields two major bands at approximately 20kd and 18kd,
as well as a minor band at 30kd. The broadness of the two
bands indicates heterogeneity caused most probably by
glycosylation, other post translational modification,
proteolytic degradation or carbamylation.

                           EXAMPLE III
Biological Activity of Bone Inductive Factor
          A rat bone formation assay according to the general
procedure of Sampath and Reddi, Proc. Natl. Acad. Sci. U.S.A.,

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80:6591-6595 (1983) is used to evaluate the osteogenic activity of the bovine bone inductive factor of the present invention obtained in Example I. This assay can also be used to evaluate bone inductive factors of other species. The ethanol precipitation step is replaced by dialyzing the fraction to be assayed against water. The solution or suspension is then redissolved in a volatile solvent, e.g. 0.1 - 0.2 % TFA, and the resulting solution added to 20mg of rat matrix. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl Acad Sci., 69:1601 (1972)] and half is fixed and processed for histological analysis. Routinely, 1um glycolmethacrylate sections are stained with Von Kossa and acid fuschin to detect new bone mineral. Alkaline phosphatase, an enzyme produced by chondroblasts and osteoblasts in the process of matrix formation, is also measured. New cartilage and bone formation often correlates with alkaline phosphatase levels. Table I below illustrates the dose response of the rat matrix samples including a control not treated with bone inductive factor.

TABLE 1

Protein* Implanted ug	Cartilage	Alk. Phos.u/l
7.5	2	Not done
2.5	3	445.7
0.83	3	77.4
0.28	0	32.5
0.00	0	31.0

*At this stage the bone inductive factor is approximately 10-15% pure.

The bone or cartilage formed is physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing as described

above, followed by autoradiography. Analysis reveals a
correlation of activity with protein bands at 28 - 30kd and a
pI 9.0. An extinction coefficient of 1 OD/mg-cm is used as an
estimate for protein and approximating the purity of bone
inductive factor in a particular fraction. In the _in vivo_ rat
bone formation assays on dilutions as described above, the
protein is active _in vivo_ at 10 to 200ng protein/gram bone to
probably greater than 1ug protein/gram bone.

                        EXAMPLE IV
Bovine Bone Inductive Factor Protein Composition
     The protein composition of Example IIA of molecular
weight 28 - 30kd is reduced as described in Example IIC and
digested with trypsin. Eight tryptic fragments are isolated
by standard procedures having the following amino acid
sequences:
Fragment 1: A A F L G D I A L D E E D L G
Fragment 2: A F Q V Q Q A A D L
Fragment 3: N Y Q D M V V E G
Fragment 4: S T P A Q D V S R
Fragment 5: N Q E A L R
Fragment 6: L S E P D P S H T L E E
Fragment 7: F D A Y Y
Fragment 8: L K P S N ? A T I Q S I V E

     A less highly purified preparation of protein from
bovine bone is prepared according to a purification scheme
similar to that described in Example I. The purification
basically varies from that previously described by omission
of the DE-52 column, the CM cellulose column and the mono S
column, as well as a reversal in the order of the
hydroxylapatite and heparin sepharose columns. Briefly, the
concentrted crude 4 M extract is brought to 85% final
concentration of ethanol at 4 degrees. The mixture is then
centrifuged, and the precipitate redissolved in 50 mM Tris,
0.15 M NaCl, 6.0 M urea. This material is then fractionated
on Heparin Sepharose as described. The Heparin bound material

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is fractionated on hydroxyapatite as described. The active fractions are pooled, concentrated, and fractionated on a high resolution gel filtration (TSK 30000 in 6 M guanidinium chloride, 50 mM Tris, pH 7.2). The active fractions are pooled, dialyzed against 0.1% TFA, and then fractionated on a C4 Vydac reverse phase column as described. The preparation is reduced and electrophoresed on an acrylamide gel. The protein corresponding to the 18K band is eluted and digested with trypsin. Tryptic fragments are isolated having the following amino acid sequences:

Fragment 9: S L K P S N H A T I Q S ? V

Fragment 10: S F D A Y Y C S ? A

Fragment 11: V Y P N M T V E S C A

Fragment 12: V D F A D I ? W

Tryptic Fragments 7 and 8 are noted to be substantially the same as Fragments 10 and 9, respectively.

A.   bBMP-1

Probes consisting of pools of oligonucleotides (or unique oligonucleotides) are designed according to the method of R. Lathe, J. Mol. Biol., 183 (1):1-12 (1985) and synthesized on an automated DNA synthesizer. One probe consists of a relatively long (32 nucleotides) "guessmer" [See J. J. Toole et al, Nature, 312:342-347 (1984)] of the following nucleotide sequence:

TCCTCATCCAGGGCAATGTCGCCCAGGAAGGC

Because the genetic code is degenerate (more than one codon can code for the same amino acid), the number of oligonucleotides in a probe pool is reduced based on the frequency of codon usage in eukaryotes, the relative stability of G:T base pairs, and the relative infrequency of the dinucleotide CpG in eukaryotic coding sequences [see Toole et al., supra.]. The second set of probes consists of shorter oligonucleotides (17 nucleotides in length) which contain all possible sequences that could encode the amino acids. The second set of probes has the following sequences:

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(a) A [A/G] [A/G] TC [T/C] TC [T/C] TC [A/G] TC [T/C] AA

(b) A [A/G] [A/G] TC [T/C] TC [T/C] TC [A/G] TCNAG

Bracketed nucleotides are alternatives. "N" means either A, T, C or G.

In both cases the regions of the amino acid sequence used for probe design are chosen by avoiding highly degenerate codons where possible. The oligonucleotides are synthesized on an automated DNA synthesizer; the probes are then radioactively labeled with polynucleotide kinase and ^{32}P-ATP.

These two sets of probes are used to screen a bovine genomic recombinant library. The library is constructed as follows: Bovine liver DNA is partially digested with the restriction endonuclease enzyme Sau 3A and sedimented through a sucrose gradient. Size fractionated DNA in the range of 15-30kb is then ligated to the bacteriophage Bam HI vector EMBL3 [Frischauf et al, _J. Mol. Biol._, 170:827-842 (1983)]. The library is plated at 8000 recombinants per plate. Duplicate nitrocellulose replicas of the plaques are made and amplified according to a modification of the procedure of Woo et al, _Proc. Natl. Acad. Sci. USA_, 75:3688-91 (1978).

The 32 mer probe is kinased with ^{32}P-gamma-ATP and hybridized to one set of filters in 5X SSC, 0.1% SDS, 5X Denhardts, 100ug/ml salmon sperm DNA at 45 degrees C and washed with 5X SSC, 0.1% SDS at 45 degrees C. The 17 mer probes are kinased and hybridized to the other set of filters in 3M tetramethylammonium chloride (TMAC), 0.1M sodium phosphate pH6.5, 1mM EDTA, 5X Denhardts, 0.6% SDS, 100ug/ml salmon sperm DNA at 48 degrees C, and washed in 3M TMAC, 50mM Tris pH8.0 at 50 degrees C. These conditions minimize the detection of mismatches to the 17 mer probe pool [see, Wood et al, _Proc. Natl. Acad. Sci, U.S.A._, 82:1585-1588 (1985)]. 400,000 recombinants are screened by this procedure and one duplicate positive is plaque purified. DNA is isolated from a plate lysate of this recombinant bacteriophage designated lambda bP-50. bP-50 was deposited December 16, 1986 with the American

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Type Culture Collection, 12301 Parklawn Drive, Rockville,
Maryland USA (hereinafter the "ATCC") under accession number
40295. This deposit as well as the other deposits contained
herein meets the requirements of the Budapest Treaty on the
International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure and Regulations thereunder.
This bp-50 clone encodes at least a portion of the bovine bone
growth factor designated bBMP-1.

The oligonucleotide hybridizing region of this bBMP-1 clone
is localized to an approximately 800bp Eco RI fragment which
is subcloned into M13 and sequenced by standard techniques.
The partial DNA sequence and derived amino acid sequence of
lambda bP-50 are shown below in Table II. The amino acid
sequences corresponding to the tryptic fragments isolated
from the bovine bone 28 to 30kd material are underlined in
Table II. The first underlined portion of the sequence
corresponds to tryptic Fragment 1 above from which the
oligonucleotide probes are designed. The second underlined
portion corresponds to tryptic Fragment 2 above. The predicted
amino acid sequence indicates that tryptic Fragment 2 is
preceded by a basic residue (R) as expected considering the
specificity of trypsin. The nucleic acid sequence preceding
the couplet CT at nucleotide positions #292-293 in Table II
is presumed to be an intron (noncoding sequence) based on the
presence of a consensus acceptor sequence (i.e., a pyrimidine
rich tract, TCTCTCTCC, followed by AG) and the lack of a
basic residue in the appropriate position of the derived
amino acid sequence. This bBMP-1 genomic sequence appears in
Table II. The presumptive bBMP-1 peptide sequence from this
genomic clone is 37 amino acids in length and is encoded by
the DNA sequence from nucleotide #294 through #404 in Table II.

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TABLE II

```
      280          290     ·  (1)            308                   323
CCTTGCCTCT TCTCTCTCCA GCT GCC TTC CTT GGG GAC ATC GCC CTG GAC GAG GAG
                         Ala Phe Leu Gly Asp Ile Ala Leu Asp Glu Glu

              338                     353                   368
GAC TTG AGG GCC TTC CAA GTG CAG CAG GCT GCG GAC CTC AGA CAG CGT GCA ACC
Asp Leu Arg Ala Phe Gln Val Gln Gln Ala Ala Asp Leu Arg Gln Arg Ala Thr

383:              398     (37)      414         424
CGC AGG TCT TCC ATC AAA GCT GCA GGTACACTGG GTACAGGCCA
Arg Arg Ser Ser Ile Lys Ala Ala
```

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B. **bBMP-2**

Two probes consisting of pools of oligonucleotides are designed on the basis of the amino acid sequence of Fragment 3 and synthesized on an automated DNA synthesizer as described above.

Probe #1: A C N A C C A T [A/G] T C [T/C] T G [A/G] A T
Probe #2: C A [A/G] G A [T/C] A T G G T N G T N G A
These probes are radioactively labeled and employed to screen the bovine genomic library constructed as described in part A except that the vector is lambda J1 Bam H1 arms [Mullins et al Nature 308: 856-858 (1984).] The radioactively labelled 17-mer Probe #1 is hybridized to the set of filters according to the method for the 17 mer probe described in part A.

400,000 recombinants are screened by the procedure described above in Part A. One duplicate positive is plaque purified and the DNA is isolated from a plate lysate of the recombinant bacteriophage designated lambda bP-21. Bacteriophage bP-21 was deposited with the ATCC under accession number ATCC 40310 on March 6, 1987. The bP-21 clone encodes the bovine growth factor designated bBMP-2.

The oligonucleotide hybridizing region of this bBMP-2 clone is localized to an approximately 1.2 kb Sac I restriction fragment which is subcloned into M13 and sequenced by standard techniques. The partial DNA sequence and derived amino acid sequence of this Sac I fragment and the contiguous Hind III-Sac I restriction fragment of bP-21 are shown below in Table III. The bBMP-2 peptide sequence from this clone is 129 amino acids in length and is encoded by the DNA sequence from nucleotide #1 through nucleotide #387. The amino acid sequence corresponding to the tryptic fragment isolated from the bovine bone 28 to 30kd material is underlined in Table III. The underlined portion of the sequence corresponds to tryptic Fragment 3 above from which the oligonucleotide probes for bBMP-2 are designed. The predicted amino acid sequence indicates that tryptic Fragment 3 is preceded by a basic

residue (K) as expected considering the specificity of trypsin. The arginine residue encoded by the CGT triplet is presumed to be the carboxy-terminus of the protein based on the presence of a stop codon (TAG) adjacent to it.

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TABLE III

```
(1)                    15                        30                          45
GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
 G   H   D   G   K   G   H   P   L   H   R   R   E   K   R

                       60                        75                          90
CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
 Q   A   K   H   K   Q   R   K   R   L   K   S   S   C   K

                       105                       120                         135
AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
 R   H   P   L   Y   V   D   F   S   D   V   G   W   N   D

                       150                       165                         180
TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
 W   I   V   A   P   P   G   Y   H   A   F   Y   C   H   G

                       195                       210                         225
GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
 E   C   P   F   P   L   A   D   H   L   N   S   T   N   H

                       240                       255                         270
GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
 A   I   V   Q   T   L   V   N   S   V   N   S   K   I   P

                       385                       300                         315
AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
 K   A   C   C   V   P   T   E   L   S   A   I   S   M   L

                       330                       345                         360
TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
 Y   L   D   E   N   E   K   V   V   L   K   N   Y   Q   D

                       375       (129)    397         407
ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA
 M   V   V   E   G   C   G   C   R

      417         427         437         447         457
TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

      467         477          487         497         507
ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

      517         527         537         547         557
AAGAAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA

      567         577         587
GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT
```

C. __bBMP-3__

    Probes consisting of pools of oligonucleotides are
designed on the basis of the amino acid sequences of the
tryptic Fragments 9 (Probe #3), 10 (Probe #2), and 11 (Probe
#1), and synthesized on an automated DNA synthesizer.
Probe #1: A C N G T C A T [A/G] T T N G G [A/G] T A

Probe #2: C A [A/G] T A [A/G] T A N G C [A/G] T C [A/G] A A

Probe #3: T G [A/G/T] A T N G T N G C [A/G] T G [A/G] T T

    A recombinant bovine genomic library constructed in
EMBL3 is screened by the TMAC hybridization procedure detailed
above in part A. 400,000 recombinants are screened in duplicate
with Probe #1 which has been labeled with ^{32}P. All
recombinants which hybridized to this probe are replated for
secondaries. Triplicate nitrocellulose replicas are made of
the secondary plates, and amplified as described. The three
sets of filters are hybridized to Probes #1, #2 and #3, again
under TMAC conditions. One clone, lambda bP-819, hybridizes
to all three probes and is plaque purified and DNA is isolated
from a plate lysate. Bacteriophage lambda bP-819 was deposited
with the ATCC on June 16, 1987 under accession number 40344.
This bP-819 clone encodes the bovine bone growth factor
designated bBMP-3.

    The region of bP-819 which hybridizes to Probe #2 is
localized and sequenced. The partial DNA and derived amino
acid sequences of this region are shown in Table IVA. The
amino acid sequences corresponding to tryptic Fragments 10
and 12 are underlined. The first underlined sequence
corresponds to Fragment 12 while the second corresponds to
Fragment 10. This region of bP-819, therefore, which hybridizes
to Probe #2 encodes at least 111 amino acids. This amino acid
sequence is encoded by the DNA sequence from nucleotide #414
through #746.

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TABLE IV. A.

```
      383         393         403         413    (1)              428
GAGGAGGAAG CGGTCTACGG GGGTCCTTCT GCCTCTGCAG  AAC AAT GAG CTT CCT GGG GCA
                                             Asn Asn Glu Leu Pro Gly Ala


             443                 458                  473                   488
GAA TAT CAG TAC AAG GAG GAT GAA GTA TGG GAG GAG AGG AAG CCT TAC AAG ACT
Glu Tyr Gln Tyr Lys Glu Asp Glu Val Trp Glu Glu Arg Lys Pro Tyr Lys Thr


                 503                 518                  533
CTT CAG ACT CAG CCC CCT GAT AAG AGT AAG AAC AAA AAG AAA CAG AGG AAG GGA
Leu Gln Thr Gln Pro Pro Asp Lys Ser Lys Asn Lys Lys Lys Gln Arg Lys Gly


             548                 563                  578                   593
CCT CAG CAG AAG AGT CAG ACG CTC CAG TTT GAT GAA CAG ACC CTG AAG AAG GCA
Pro Gln Gln Lys Ser Gln Thr Leu Gln Phe Asp Glu Gln Thr Leu Lys Lys Ala


             608                 623                  638
AGA AGA AAG CAA TGG ATT GAA CCC CGG AAT TGT GCC AGA CGG TAC CTT AAA GTG
Arg Arg Lys Gln Trp Ile Glu Pro Arg Asn Cys Ala Arg Arg Tyr Leu Lys Val


653                 668                  683                   698
GAC TTC GCA GAT ATT GGC TGG AGC GAA TGG ATT ATT TCC CCC AAG TCC TTC GAT
Asp Phe Ala Asp Ile Gly Trp Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp


          713                 728              743  (111)       756
GCC TAT TAC TGC TCC GGA GCG TGC CAG TTC CCC ATG CCA AAG   GTAGCCATTG
Ala Tyr Tyr Cys Ser Gly Ala Cys Gln Phe Pro MET Pro Lys


          766         776         786
TTTTTTGTCC TGTCCTTCCC ATTTCCATAG
```

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The region of bP-819 which hybridizes to Probe #1 and #3 is localized and sequenced. The partial DNA and derived amino acid sequences of this region are shown in Table IVB. The amino acid sequences corresponding to tryptic Fragments 9 and 11 are underlined. The first underlined sequence corresponds to Fragment 9 while the second underlined sequence corresponds to Fragment 11. The peptide sequence of this region of bP-819 which hybridizes to Probe #1 and #3 is 64 amino acids in length encoded by nucleotide #305 through #493 of Table IVB. The arginine residue encoded by the AGA triplet is presumed to be the carboxy-terminus of the protein based on the presence of a stop codon (TAA) adjacent to it. The nucleic acid sequence preceding the couplet TC (positions 305-306) is presumed to be an intron (non-coding sequence) based on the presence of a consensus acceptor sequence (i.e. a pyrimidine-rich stretch, TTCTCCCTTTTCGTTCCT, followed by AG) and the presence of a stop rather than a basic residue in the appropriate position of the derived amino acid sequence.

bBMP-3 is therefore characterized by the DNA and amino acid sequence of Table IV A and Table IV B. The peptide sequence of this clone is 175 amino acids in length and is encoded by the DNA sequence from nucleotide #414 through nucleotide #746 of Table IV A and nucleotide #305 through nucleotide #493 of Table IV B.

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TABLE IV. B.

```
      284        294        304   (112)            319
CTAACCTGTG TTCTCCCTTT TCGTTCCTAG TCT TTG AAG CCA TCA AAT CAC GCT ACC
                                 Ser Leu Lys Pro Ser Asn His Ala Thr


334                349                    364           379
ATC CAG AGT ATA GTG AGA GCT GTG GGG GTC GTC CCT GGA ATC CCC GAG CCT TGC
Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile Pro Glu Pro Cys


           394                409                424                 439
TGT GTG CCA GAA AAG ATG TCC TCA CTC AGC ATC TTA TTC TTT GAT GAA AAC AAG
Cys Val Pro Glu Lys MET Ser Ser Leu Ser Ile Leu Phe Phe Asp Glu Asn Lys


               454                469                484     (175)
AAT GTG GTA CTT AAA GTA TAT CCA AAC ATG ACA GTA GAG TCT TGT GCT TGC AGA
Asn Val Val Leu Lys Val Tyr Pro Asn MET Thr Val Glu Ser Cys Ala Cys Arg


      503        513        523        533
TAACCTGGTG AAGAACTCAT CTGGATGCTT AACTCAATCG
```

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EXAMPLE V

Human Bone Inductive Factors

A. hBMP-1

Because the bovine and human bone growth factor genes
are presumed to be significantly homologous, the bovine bBMP-
1 DNA sequence of Table II (or portions thereof) is used as a
probe to screen a human ge.:omic library. The 800bp EcoRI
fragment of the bovine genomic clone is labeled with ^{32}P by
nick-translation. A human genomic library (Toole et al.,
supra) is plated on 20 plates at 40,000 recombinants per
plate. Duplicate nitrocellulose filter replicas are made of
each plate and hybridized to the nick-translated probe in 5 X
SSC, 5 X Denhardt's, 100ug/ml denatured salmon sperm DNA,
0.1% SDS (the standard hybridization solution) at 50 degrees
centigrade for approximately 14 hours. The filters are then
washed in 1 X SSC, 0.1% SDS at 50 degrees centigrade and
subjected· to autoradiography. Five duplicate positives are
isolated and plaque purified. DNA is obtained from a plate
lysate of one of these recombinant bacteriophage, designated
LP-H1. LP-H1 was deposited with the ATCC on March 6, 1987 under
accession number 40311. This clone encodes at least a portion
of the human genomic bone growth factor called hBMP-1. The
hybridizing region of LP-H1 is localized to a 2.5kb XbaI/HindIII
restriction fragment.

The partial DNA sequence and derived amino acid
sequence of lambda LP-H1 are shown below in Table V.   The
peptide sequence from this clone is 37 amino acids in length
and is encoded by the DNA sequence from nucleotide #3440 through
nucleotide #3550. The coding sequence of Table V is flanked
by approximately 28 nucleotides (a presumptive 5' noncoding
sequence) as well as approximately 19 nucleotides (a presumptive
3' noncoding sequence. A comparison of the bBMP-1 sequence
of Table II with the hBMP-1 genomic sequence of Table V
indicates the significant homology between the two.

Because the size of coding regions and the positions

of noncoding regions is generally conserved in homologous genes of different species, the locations of the coding and noncoding regions of the bone inductive factor genes may be identified. Regions of homology between the two species' genes, flanked by RNA processing signals at homologous sites, indicate a coding region.

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TABLE V

```
     3419        3429        3439  (1)              3454
CAGCCCTGGC TTCTTCTTTT CTCTTTAGCT GCC TTT CTT GGG GAC ATT GCC CTG GAC
                                 Ala Phe Leu Gly Asp Ile Ala Leu Asp

3469                 3484                 3499                 3514
GAA GAG GAC CTG AGG GCC TTC CAG GTA CAG CAG GCT GTG GAT CTC AGA CGG CAC
Glu Glu Asp Leu Arg Ala Phe Gln Val Gln Gln Ala Val Asp Leu Arg Arg His

        3529                 3544  (37)   3560        3570
ACA GCT CGT AAG TCC TCC ATC AAA GCT GCA GGTAAGCCGG GTGCCAATGG
Thr Ala Arg Lys Ser Ser Ile Lys Ala Ala
```

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A probe specific for the human coding sequence given in Table V is used to identify a human cell line or tissue which synthesizes bone inductive factor. The probe is made according to the following method. Two oligonucleotides having the following sequences:

(a) GGGAATTCTGCCTTTCTTGGGGACATTGCCCTGGACGAAGAGGACCTGAG

(b) CGGGATCCGTCTGAGATCCACAGCCTGCTGTACCTGGAAGGCCCTCAGG

are synthesized on an automated synthesizer, annealed, extended using the Klenow fragment of E. coli DNA polymerase I, digested with the restriction enzymes Eco RI and Bam HI, and inserted into an M13 vector. A single-stranded ^{32}P-labeled probe is then from template preparation of this subclone by standard techniques. Polyadenylated RNAs from various cell and tissue sources are electrophoresed on formaldehyde-agarose gels and transfered to nitrocellulose by the method of Toole et. al., supra. The probe is then hybridized to the nitrocellulose blot in 50% formamide, 5 X SSC, 0.1% SDS, 40 mM sodium phosphate pH 6.5, 100 ug/ml denatured salmon sperm DNA, and 5 mM vanadyl ribonucleosides at 42° C overnight and washed at 65° C in 0.2 X SSC, 0.1% SDS. Following autoradiography, the lane containing RNA from the human osteosarcoma cell line U-2 OS contains hybridizing bands corresponding to RNA species of approximately 4.3 and 3.0 kb.

cDNA is synthesized from U-2 OS polyadenylated RNA and cloned into lambda gt10 by established techniques (Toole et al., supra). 20,000 recombinants from this library are plated on each of 50 plates. Duplicate nitrocellulose replicas are made of the plates. The above described oligonucleotides are kinased with ^{32}P-gamma-ATP and hybridized to the two sets of replicas at 55° centigrade in standard hybridization solution overnight. The filters are then washed in 1 X SSC, 0.1% SDS at 55° centigrade and subjected to autoradiography. One duplicate positive, designated lambda U2OS-1, is plaque purified. Lambda U2OS-1 was deposited with the ATCC on June 16, 1987 under accession number 40343.

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The entire nucleotide sequence and derived amino acid sequence of the insert of lambda U2OS-1 is given in Table VI. This cDNA clone encodes a Met followed by a hydrophobic leader sequence characteristic of a secreted protein, and contains a stop codon at nucleotide positons 2226 - 2228. This clone contains an open reading frame of 2190bp, encoding a protein of 730 amino acids with a molecular weight og 83kd based on this amino acid sequence. The clone contains sequence identical to the coding region given in Table V. This protein is contemplated to represent a primary translation product which is cleaved upon secretion to produce the hBMP-1 protein. This clone is therefore a cDNA for hBMP-1 corresponding to human gene fragment contained in the genomic hBMP-1 sequence lambda LP-H1. It is noted that amino acids #550 to #590 of BMP-1 are homologous to epidermal growth factor and the "growth factor" domains of Protein C, Factor X and Factor IX.

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TABLE VI

```
         10          20          30       (1)                50
CTAGAGGCCG CTTCCCTCGC CGCCGCCCCG CCAGC ATG CCC GGC GTG GCC CGC CTG CCG
                                        MET Pro Gly Val Ala Arg Leu Pro


         65              80              95                 110
CTG CTG CTC GGG CTG CTG CTC CTC CCG CGT CCC GGC CGG CCG CTG GAC TTG GCC
Leu Leu Leu Gly Leu Leu Leu Leu Pro Arg Pro Gly Arg Pro Leu Asp Leu Ala


             125             140             155
GAC TAC ACC TAT GAC CTG GCG GAG GAG GAC GAC TCG GAG CCC CTC AAC TAC AAA
Asp Tyr Thr Tyr Asp Leu Ala Glu Glu Asp Asp Ser Glu Pro Leu Asn Tyr Lys


170             185             200             215
GAC CCC TGC AAG GCG GCT GCC TTT CTT GGG GAC ATT GCC CTG GAC GAA GAG GAC
Asp Pro Cys Lys Ala Ala Ala Phe Leu Gly Asp Ile Ala Leu Asp Glu Glu Asp


         230             245                 260             275
CTG AGG GCC TTC CAG GTA CAG CAG GCT GTG GAT CTC AGA CGG CAC ACA GCT CGT
Leu Arg Ala Phe Gln Val Gln Gln Ala Val Asp Leu Arg Arg His Thr Ala Arg


             290             305             320
AAG TCC TCC ATC AAA GCT GCA GTT CCA GGA AAC ACT TCT ACC CCC AGC TGC CAG
Lys Ser Ser Ile Lys Ala Ala Val Pro Gly Asn Thr Ser Thr Pro Ser Cys Gln


     335             350             365             380
AGC ACC AAC GGG CAG CCT CAG AGG GGA GCC TGT GGG AGA TGG AGA GGT AGA TCC
Ser Thr Asn Gly Gln Pro Gln Arg Gly Ala Cys Gly Arg Trp Arg Gly Arg Ser


         395             410             425
CGT AGC CGG CGG GCG GCG ACG TCC CGA CCA GAG CGT GTG TGG CCC GAT GGG GTC
Arg Ser Arg Arg Ala Ala Thr Ser Arg Pro Glu Arg Val Trp Pro Asp Gly Val


440             455             470             485
ATC CCC TTT GTC ATT GGG GGA AAC TTC ACT GGT AGC CAG AGG GCA GTC TTC CGG
Ile Pro Phe Val Ile Gly Gly Asn Phe Thr Gly Ser Gln Arg Ala Val Phe Arg


         500             515             530             545
CAG GCC ATG AGG CAC TGG GAG AAG CAC ACC TGT GTC ACC TTC CTG GAG CGC ACT
Gln Ala MET Arg His Trp Glu Lys His Thr Cys Val Thr Phe Leu Glu Arg Thr


             560             575             590
GAC GAG GAC AGC TAT ATT GTG TTC ACC TAT CGA CCT TGC GGG TGC TGC TCC TAC
Asp Glu Asp Ser Tyr Ile Val Phe Thr Tyr Arg Pro Cys Gly Cys Cys Ser Tyr


     605             620             635             650
GTG GGT CGC CGC GGC GGG GGC CCC CAG GCC ATC TCC ATC GGC AAG AAC TGT GAC
Val Gly Arg Arg Gly Gly Gly Pro Gln Ala Ile Ser Ile Gly Lys Asn Cys Asp
```

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```
            665                    680                    695
AAG TTC GGC ATT GTG GTC CAC GAG CTG GGC CAC GTC GTC GGC TTC TGG CAC GAA
Lys Phe Gly Ile Val Val His Glu Leu Gly His Val Val Gly Phe Trp His Glu


710                    725                    740                    755
CAC ACT CGG CCA GAC CGG GAC CGC CAC GTT TCC ATC GTT CGT GAG AAC ATC CAG
His Thr Arg Pro Asp Arg Asp Arg His Val Ser Ile Val Arg Glu Asn Ile Gln


            770                    785                    800                    815
CCA GGG CAG GAG TAT AAC TTC CTG AAG ATG GAG CCT CAG GAG GTG GAG TCC CTG
Pro Gly Gln Glu Tyr Asn Phe Leu Lys MET Glu Pro Gln Glu Val Glu Ser Leu


            830                    845                    860
GGG GAG ACC TAT GAC TTC GAC AGC ATC ATG CAT TAC GCT CGG AAC ACA TTC TCC
Gly Glu Thr Tyr Asp Phe Asp Ser Ile MET His Tyr Ala Arg Asn Thr Phe Ser


            875                    890                    905                    920
AGG GGC ATC TTC CTG GAT ACC ATT GTC CCC AAG TAT GAG GTG AAC GGG GTG AAA
Arg Gly Ile Phe Leu Asp Thr Ile Val Pro Lys Tyr Glu Val Asn Gly Val Lys


            935                    950                    965
CCT CCC ATT GGC CAA AGG ACA CGG CTC AGC AAG GGG GAC ATT GCC CAA GCC CGC
Pro Pro Ile Gly Gln Arg Thr Arg Leu Ser Lys Gly Asp Ile Ala Gln Ala Arg


980                    995                    1010                   1025
AAG CTT TAC AAG TGC CCA GCC TGT GGA GAG ACC CTG CAA GAC AGC ACA GGC AAC
Lys Leu Tyr Lys Cys Pro Ala Cys Gly Glu Thr Leu Gln Asp Ser Thr Gly Asn


            1040                   1055                   1070                   1085
TTC TCC TCC CCT GAA TAC CCC AAT GGC TAC TCT GCT CAC ATG CAC TGC GTG TGG
Phe Ser Ser Pro Glu Tyr Pro Asn Gly Tyr Ser Ala His MET His Cys Val Trp


            1100                   1115                   1130
CGC ATC TCT GTC ACA CCC GGG GAG AAG ATC ATC CTG AAC TTC ACG TCC CTG GAC
Arg Ile Ser Val Thr Pro Gly Glu Lys Ile Ile Leu Asn Phe Thr Ser Leu Asp


1145                   1160                   1175                   1190
CTG TAC CGC AGC CGC CTG TGC TGG TAC GAC TAT GTG GAG GTC CGA GAT GGC TTC
Leu Tyr Arg Ser Arg Leu Cys Trp Tyr Asp Tyr Val Glu Val Arg Asp Gly Phe


            1205                   1220                   1235
TGG AGG AAG GCG CCC CTC CGA GGC CGC TTC TGC GGG TCC AAA CTC CCT GAG CCT
Trp Arg Lys Ala Pro Leu Arg Gly Arg Phe Cys Gly Ser Lys Leu Pro Glu Pro


1250                   1265                   1280                   1295
ATC GTC TCC ACT GAC AGC CGC CTC TGG GTT GAA TTC CGC AGC AGC AGC AAT TGG
Ile Val Ser Thr Asp Ser Arg Leu Trp Val Glu Phe Arg Ser Ser Ser Asn Trp


            1310                   1325                   1340                   1355
GTT GGA AAG GGC TTC TTT GCA GTC TAC GAA GCC ATC TGC GGG GGT GAT GTG AAA
Val Gly Lys Gly Phe Phe Ala Val Tyr Glu Ala Ile Cys Gly Gly Asp Val Lys
```

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```
                1370                  1385                  1400
AAG GAC TAT GGC CAC ATT CAA TCG CCC AAC TAC CCA GAC GAT TAC CGG CCC AGC
Lys Asp Tyr Gly His Ile Gln Ser Pro Asn Tyr Pro Asp Asp Tyr Arg Pro Ser


                1415                  1430                  1445                  1460
AAA GTC TGC ATC TGG CGG ATC CAG GTG TCT GAG GGC TTC CAC GTG GGC CTC ACA
Lys Val Cys Ile Trp Arg Ile Gln Val Ser Glu Gly Phe His Val Gly Leu Thr


                1475                  1490                  1505
TTC CAG TCC TTT GAG ATT GAG CGC CAC GAC AGC TGT GCC TAC GAC TAT CTG GAG
Phe Gln Ser Phe Glu Ile Glu Arg His Asp Ser Cys Ala Tyr Asp Tyr Leu Glu


        1520                  1535                  1550                  1565
GTG CGC GAC GGG CAC AGT GAG AGC AGC ACC CTC ATC GGG CGC TAC TGT GGC TAT
Val Arg Asp Gly His Ser Glu Ser Ser Thr Leu Ile Gly Arg Tyr Cys Gly Tyr


                1580                  1595                  1610                  1625
GAG AAG CCT GAT GAC ATC AAG AGC ACG TCC AGC CGC CTC TGG CTC AAG TTC GTC
Glu Lys Pro Asp Asp Ile Lys Ser Thr Ser Ser Arg Leu Trp Leu Lys Phe Val


                1640                  1655                  1670
TCT GAC GGG TCC ATT AAC AAA GCG GGC TTT GCC GTC AAC TTT TTC AAA GAG GTG
Ser Asp Gly Ser Ile Asn Lys Ala Gly Phe Ala Val Asn Phe Phe Lys Glu Val


        1685                  1700                  1715                  1730
GAC GAG TGC TCT CGG CCC AAC CGC GGG GGC TGT GAG CAG CGG TGC CTC AAC ACC
Asp Glu Cys Ser Arg Pro Asn Arg Gly Gly Cys Glu Gln Arg Cys Leu Asn Thr


                1745                  1760                  1775
CTG GGC AGC TAC AAG TGC AGC TGT GAC CCC GGG TAC GAG CTG GCC CCA GAC AAG
Leu Gly Ser Tyr Lys Cys Ser Cys Asp Pro Gly Tyr Glu Leu Ala Pro Asp Lys


        1790                  1805                  1820                  1835
CGC CGC TGT GAG GCT GCT TGT GGC GGA TTC CTC ACC AAG CTC AAC GGC TCC ATC
Arg Arg Cys Glu Ala Ala Cys Gly Gly Phe Leu Thr Lys Leu Asn Gly Ser Ile


                1850                  1865                  1880                  1895
ACC AGC CCG GGC TGG CCC AAG GAG TAC CCC CCC AAC AAG AAC TGC ATC TGG CAG
Thr Ser Pro Gly Trp Pro Lys Glu Tyr Pro Pro Asn Lys Asn Cys Ile Trp Gln


                1910                  1925                  1940
CTG GTG GCC CCC ACC CAG TAC CGC ATC TCC CTG CAG TTT GAC TTC TTT GAG ACA
Leu Val Ala Pro Thr Gln Tyr Arg Ile Ser Leu Gln Phe Asp Phe Phe Glu Thr


        1955                  1970                  1985                  2000
GAG GGC AAT GAT GTG TGC AAG TAC GAC TTC GTG GAG GTG CGC AGT GGA CTC ACA
Glu Gly Asn Asp Val Cys Lys Tyr Asp Phe Val Glu Val Arg Ser Gly Leu Thr


        2015                  2030                  2045
GCT GAC TCC AAG CTG CAT GGC AAG TTC TGT GGT TCT GAG AAG CCC GAG GTC ATC
Ala Asp Ser Lys Leu His Gly Lys Phe Cys Gly Ser Glu Lys Pro Glu Val Ile
```

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  2060                 2075                 2090                 2105
ACC TCC CAG TAC AAC AAC ATG CGC GTG GAG TTC AAG TCC GAC AAC ACC GTG TCC
Thr Ser Gln Tyr Asn Asn MET Arg Val Glu Phe Lys Ser Asp Asn Thr Val Ser


              2120                 2135                 2150                 2165
AAA AAG GGC TTC AAG GCC CAC TTC TTC TCA GAA AAG AGG CCA GCT CTG CAG CCC
Lys Lys Gly Phe Lys Ala His Phe Phe Ser Glu Lys Arg Pro Ala Leu Gln Pro


                     2180                 2195                 2210
CCT CGG GGA CGC CCC CAC CAG CTC AAA TTC CGA GTG CAG AAA AGA AAC CGG ACC
Pro Arg Gly Arg Pro His Gln Leu Lys Phe Arg Val Gln Lys Arg Asn Arg Thr
    (730)
    2225      2235      2245      2255      2265      2275      2285
CCC CAG TGAGGCCTGC CAGGCCTCCC GGACCCCTTG TTACTCAGGA ACCTCACCTT GGACGGAATG
Pro Gln


      2295      2305      2315      2325      2335      2345      2355
GGATGGGGGC TTCGGTGCCC ACCAACCCCC CACCTCCACT CTGCCATTCC GGCCCACCTC CCTCTGGCCG


      2365      2375      2385      2395      2405      2415      2425
GACAGAACTG GTGCTCTCTT CTCCCCACTG TGCCCGTCCG CGGACCGGGG ACCCTTCCCC GTGCCCTACC


      2435      2445      2455      2465      2475      2485      2495
CCCTCCCATT TTCATGGTGT CTGTGACATT TCCTGTTGTG AAGTAAAAGA GGGACCCCTG CGTCCTGCCT


CTAGA
```

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B.   hBMP-2: Class I and II

The HindIII-SacI bovine genomic bBMP-2 fragment
described in Example IV B. is subcloned into an M13 vector.
A ^{32}P-labeled single-stranded DNA probe is made from a template
preparation of this subclone. This probe is used to screen
polyadenylated RNAs from various cell and tissue sources as
described above in part A. A hybridizing band corresponding
to an mRNA species of approximately 3.8 kb is detected in the
lane containing RNA from the human cell line U-2 OS. The
HindIII-SacI fragment is labeled with ^{32}P by nick translation
and used to screen the nitrocellulose filter replicas of the
above-described U-2 OS cDNA library by hybridization in
standard hybridization buffer at 65^{o} overnight followed by
washing in 1 X SSC, 0.1% SDS at 65^{o}. Twelve duplicate positive
clones are picked and replated for secondaries. Duplicate
nitrocellulose replicas are made of the secondary plates and
both sets hybridized to the bovine genomic probe as the
primary screening was performed. One set of filters is then
washed in 1 X SSC, 0.1% SDS; the other in 0.1 X SSC, 0.1% SDS
at 65^{o}.

Two classes of hBMP-2 cDNA clones are evident based
on strong (4 recombinants) or weak (7 recombinants)
hybridization signals under the more stringent washing
conditions (0.1 X SSC, 0.1% SDS). All 11 recombinant
bacteriophage are plaque purified, small scale DNA preparations
made from plate lysates of each, and the inserts subcloned
into pSP65 and into M13 for sequence analysis. Sequence
analysis of the strongly hybridizing clones designated hBMP-2
Class I (also known as BMP-2) indicates that they have extensive
sequence homology with the sequence given in Table III.
These clones are therefore cDNA encoding the human equivalent
of the protein encoded by the bBMP-2 gene whose partial sequence
is given in Table III. Sequence analysis of the weakly
hybridizing recombinants designated hBMP-2 Class II (also
known as BMP-4) indicates that they are also quite homologous

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with the sequence giv n in Table III at the 3' end of their
coding regions, but less so in the more 5' regions. Thus
they encode a human protein of similar, though not identical,
structure to that above.

Full length hBMP-2 Class I cDNA clones are obtained
in the following manner. The 1.5 kb insert of one of the
Class II subclones (II-10-1) is isolated and radioactively
labeled by nick-translation. One set of the nitrocellulose
replicas of the U-2 OS cDNA library screened above (50 filters,
corresponding to 1,000,000 recombinant bacteriophage) are
rehybridized with this probe under stringent conditions
(hybridization at 65° in standard hybridization buffer;
washing at 65° in 0.2 X SSC, 0.1% SDS). All recombinants
which hybridize to the bovine genomic probe which do not
hybridize to the Class II probe are picked and plaque purified
(10 recombinants). Plate stocks are made and small scale
bacteriophage DNA preparations made. After subcloning into
M13, sequence analysis indicates that 4 of these represent
clones which overlap the original Class I clone. One of
these, lambda U2OS-39, contains an approximately 1.5 kb
insert and was deposited with the ATCC on June 16, 1987
under accession number 40345. The partial DNA sequence
(compiled from lambda U2OS-39 and several other hBMP-2 Class
I cDNA recombinants) and derived amino acid sequence are
shown below in Table VII. Lambda U2OS-39 is expected to
contain all of the nucleotide sequence necessary to encode
the entire human counterpart of the protein BMP-2 Class II
encoded by the bovine gene segment whose partial sequence is
presented in Table III. This human cDNA hBMP-2 Class II
contains an open reading frame of 1188 bp, encoding a protein
of 396 amino acids. This protein of 396 amino acids has a
molecular weight of 45kd based on this amino acid sequence.
It is contemplated that this sequence represents the primary
translation product. The protein is preceded by a 5'
untranslated region of 342 bp with stop codons in all frames.

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The 13 bp region preceding this 5' untranslated region
represents a linker used in the cDNA cloning procedure.

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TABLE VII

```
          10          20          30          40          50          60          70
   GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT AACTTGCGCA


          80          90         100         110         120         130         140
   CCCCACTTTG CGCCGGTGCC TTTGCCCCAG CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCTCC


         150         160         170       · 180         190         200         210
   ACTCCTCGGC CTTGCCCGAC ACTGAGACGC TGTTCCCAGC GTGAAAAGAG AGACTGCGCG GCCGGCACCC


         220         230         240         250         260         270         280
   GGGAGAAGGA· GGAGGCAAAG AAAAGGAACG GACATTCGGT CCTTGCGCCA GGTCCTTTGA CCAGAGTTTT


         290         300         310         320         330         340         350
   TCCATGTGGA CGCTCTTTCA ATGGACGTGT CCCCGCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT
```

```
      (1)          370                  385                  400
   CGACC ATG GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC
         MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val

                  415                  430                  445
   CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG
   Leu Leu Gly Gly·Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala

   460                  475                  490                · 505
   GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG
   Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu

          520                  535                  550                  565
   TTC GAG TTG CGG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC
   Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser

          580                  595                  610
   AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCG GGT
   Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly

          625                  640                  655                  670
   CAG CCG GGC TCA CCC GCC CCA GAC CAC CGG TTG GAG AGG GCA GCC AGC CGA GCC
   Gln Pro Gly Ser Pro·Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala

          685                  700                  715
   AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG
   Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr
```

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730                 745                 760                 775
AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu


        790                 805                 820                 835
GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala


        850                 865                 880
TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys


        895                 910                 925                 940
CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu


        955                 970                 985
GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET


1000                1015                1030                1045
CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC
Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His


        1060                1075                1090                1105
TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu


        1120                1135                1150
CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly


   1165                 1180                1195                1210
CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC
His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His


        1225                1240                1255
AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC
Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp


1270                1285                1300                 1315
TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC
Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala


        1330                1345                1360                1375
TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT
Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr


        1390                1405                1420
AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG
Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys
```

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      1435                1450                1465                1480
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu

            1495                1510                1525
AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

1540(396)   1553      1563      1573      1583      1593      1603
TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
Cys Arg:


AAAA.
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Full-length hBMP-2Class II human cDNA clones are
obtained in the following manner. The 200 bp EcoRI-SacI
fragment from the 5' end of the Class II recombinant II-10-1
is isolated from its plasmid subclone, labeled by nick-
translation, and hybridized to a set of duplicate nitrocellulose
replicas of the U-2 OS cDNA library (25 filters/set;
representing 500,000 recombinants). Hybridization and
washing are performed under stringent conditions as described
above. 16 duplicate positives are picked and replated for
secondaries. Nitrocellulose filter replicas of the secondary
plates are made and hybridized to an oligonucleotide which
was synthesized to correspond to the sequence of II-10-1 and
is of the following sequence:

CGGGCGCTCAGGATACTCAAGACCAGTGCTG

Hybridization is in standard hybridization buffer AT 50° C
with washing at 50° in 1 X SSC, 0.1% SDS. 14 recombinant
bacteriophage which hybridize to this oligonucleotide are
plaque purified. Plate stocks are made and small scale
bacteriophage DNA preparations made. After sucloning 3 of
these into M13, sequence analysis indicates that they represent
clones which overlap the original Class II clone. One of
these, lambda U2OS-3, was deposited with the ATCC under
accession number 40342 on June 16, 1987. U2OS-3 contains an
insert of approximately 1.8 kb. The partial DNA sequence and
derived amino acid sequence of U2OS-3 are shown below in
Table VIII. This clone is expected to contain all of the
nucleotide sequence necessary to encode the entire human BMP-
2 Class II protein. This cDNA contains an open reading frame
of 1224 bp, encoding a protein of 408 amino acids, preceded
by a 5' untranslated region of 394 bp with stop codons in all
frames, and contains a 3' untranslated region of 308 bp
following the in-frame stop codon. The 8 bp region preceding
the 5' untranslated region represents a linker used in the
cDNA cloning procedure. This protein of 408 amino acids has
molecular weight of 47kd and is contemplated to represent the

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primary translation product.

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TABLE VIII

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        10           20           30           40           50           60           70
CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG CAAGGAGCGC GGAGCCCGGC CCGGAAGCTA GGTGAGTGTG


        80           90          100          110          120          130          140
GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC


       150          160          170          180          190          200          210
GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC AGGTTCACTG


       220          230          240          250          260          270          280
CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC


       290          300          310          320          330          340          350
GTAGTGCCAT CCCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG


       360          370          380          390          400          (1)
CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT
                                                            MET Ile Pro


      417                         432                         447                         462
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala


                  477                         492                         507
AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln


  522                         537                         552                         567
GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe


      582                         597                         612                         627
GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys


              642                         657                         672
AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu


      687                         702                         717                         732
GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC
Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala
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              747                      762                      777
AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile


  792                      807                      822                      837
CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile


              852                      867                      882                      897
CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG
Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val


                    912                      927                      942
GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val


  957                      972                      987                      1002
ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp


              1017                     1032                     1047
ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT
Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro


  1062                     1077                     1092                     1107
GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG
Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu


              1122                     1137                     1152                     1167
GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser


              1182                     1197                     1212
CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC
Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val


  1227                     1242                     1257                     1272
ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG
Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys


              1287                     1302                     1317
CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg


1332                     1347                     1362                     1377
CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val


              1392                     1407                     1422                     1437
GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
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        1452                 1467                 1482
GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser


    1497                 1512                    1527                1542
GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile


        1557                 1572                1587
TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu


1602          1617        (408)  1636         1646        1656
ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
MET Val Val Glu Gly Cys Gly Cys Arg


    1666       1676       1686       1696       1706       1716       1726
ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC


    1736       1746       1756       1766       1776       1786       1796
ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC


    1806       1816       1826       1836       1846       1856       1866
ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA


    1876       1886       1896       1906       1916       1926       1936
ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAAACT


    1946
CTAGAGTCGA CGGAATTC
```

The sequences of BMP-2 Class I and II, as well as
BMP-3 as shown in Tables III, IV, VII and VIII have significant
homology to the beta (B) and beta (A) subunits of the inhibins.
The inhibins are a family of hormones which are presently
being investigated for use in contraception. See, A. J.
Mason et al, _Nature_, 318:659-663 (1985). To a lesser extent
they are also homologous to Mullerian inhibiting substance
(MIS), a testicular glycoprotein that causes regression of
the Mullerian duct during development of the male embryo and
transforming growth factor-beta (TGF-b) which can inhibit or
stimulate growth of cells or cause them to differentiate.
Furthermore, the sequence of Table VII encoding hBMP-2 Class
II has significant homology to the _Drosophila_ decapentaplegic
(DPP-C) locus transcript. See, J. Massague, _Cell_, 49:437-438
(1987); R. W. Padgett et al, _Nature_, 325:81-84 (1987); R. L.
Cate et al, _Cell_ 45: 685-698 (1986). It is considered possible
therefore that BMP-2 Class II is the human homolog of the
protein made from this transcript from this developmental mutant
locus.

C. __BMP-3__

Because bovine and human bone growth factor genes are
presumed to be significantly homologous, oligonucleotide
probes which have been shown to hybridize to the bovine DNA
sequence of Table IV.A and IV.B are used to screen a human
genomic library· A human genomic library (Toole et al.,
supra) is screened using these probes, and presumptive positives
are isolated and DNA sequence obtained as described above.
Evidence that this recombinant encodes a portion of the human
bone inductive factor molecule relies on the bovine/human
protein and gene structure homologies.

Once a recombinant bacteriophage containing DNA
encoding a portion of the human BMP-3 molecule is obtained
the human coding sequence is used as a probe as described in
Example V (A) to identify a human cell line or tissue which
synthesizes BMP-3. mRNA is selected by oligo (dT) cellulose

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chromatography and cDNA is synthesized and cloned in lambda gtl0 by established techniques (Toole et al., supra).

Alternatively, the entire gene encoding this human bone inductive factor can be identified and obtained in additional recombinant clones if necessary. Additional recombinants containing further 3' or 5' regions of this human bone inductive factor gene can be obtained by identifying unique DNA sequences at the end(s) of the original clone and using these as probes to rescreen the human genomic library. The gene can then be reassembled in a single plasmid by standard molecular biology techniques and amplified in bacteria. The entire human BMP-3 factor gene can then be transferred to an appropriate expression vector. The expression vector containing the gene is then transfected into a mammalian cell, e.g. monkey COS cells, where the human gene is transcribed and the RNA correctly spliced. Media from the transfected cells are assayed for bone inductive factor activity as described herein as an indication that the gene is complete. mRNA is obtained from these cells and cDNA synthesized from this mRNA source and cloned. The procedures described above may similarly be employed to isolate other species' bone inductive factor of interest by utilizing the bovine bone inductive factor and/or human bone inductive factor as a probe source. Such other species' bone inductive factor may find similar utility in, inter alia, fracture repair.

<u>EXAMPLE VI</u>

<u>Expression of Bone Inductive Factors.</u>

In order to produce bovine, human or other mammalian bone inductive factors, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells by conventional genetic engineering techniques.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Tables II-

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VIII or other modified sequences and known vectors, such as
pCD [Okayama et al., Mol. Cell Biol., 2:161-170 (1982)] and
pJL3; pJL4 [Gough et al., EMBO J., 4:645-653 (1985)]. The
transformation of these vectors into appropriate host cells
can result in expression of osteoinductive factors. One
skilled in the art could manipulate the sequences of Tables
II-VIII by eliminating or replacing the mammalian regulatory
sequences flanking the coding sequence with bacterial sequences
to create bacterial vectors for intracellular or extracellular
expression by bacterial cells. For example, the coding
sequences could be further manipulated (e.g. ligated to other
known linkers or modified by deleting non-coding sequences
there-from or altering nucleotides therein by other known
techniques). The modified bone inductive factor coding
sequence could then be inserted into a known bacterial vector
using procedures such as described in T. Taniguchi et al.,
Proc. Natl Acad. Sci. USA, 77:5230-5233 (1980). This exemplary
bacterial vector could then be transformed into bacterial
host cells and bone inductive factor expressed thereby. For
a strategy for producing extracellular expression of bone
inductive factor in bacterial cells., see, e.g. European
patent application EPA 177,343.

Similar manipulations can be performed for the
construction of an insect vector [See, e.g. procedures described
in published European patent application 155,476] for expression
in insect cells. A yeast vector could also be constructed
employing yeast regulatory sequences for intracellular or
extracellular expression of the factors of the present invention
by yeast cells. [See, e.g., procedures described in published
PCT application WO86/00639 and European patent application EPA
123,289].

A method for producing high levels of an osteoinductive
factor of the invention from mammalian cells involves the
construction of cells containing multiple copies of the
heterologous bone inductive factor gene. The heterologous gene

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can be linked to an amplifiable marker, e.g. the dihydrofolate
reductase (DHFR) gene for which cells containing increased gene
copies can be selected for propagation in increasing
concentrations of methotrexate (MTX) according to the procedures
of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982). This
approach can be employed with a number of different cell types.

For example, a plasmid containing a DNA sequence for
a bone inductive factor of the invention in operative
association with other plasmid sequences enabling expression
thereof and the DHFR expression plasmid pAdA26SV(A)3 [Kaufman
and Sharp, Mol. Cell. Biol., 2:1304 (1982)] can be co-introduced
into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate
coprecipitation and transfection. DHFR expressing transformants
are selected for growth in alpha media with dialyzed fetal
calf serum, and subsequently selected for amplification by
growth in increasing concentrations of MTX (sequential steps
in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et
al., Mol Cell Biol., 5:1750 (1983). Transformants are cloned,
and biologically active bone inductive factor expression is
monitored by rat bone formation assay. Bone inductive factor
expression should increase with increasing levels of MTX
resistance. Similar procedures can be followed to produce
other bone inductive factors.

Alternatively, the human gene is expressed directly,
as described above. Active bone inductive factor may be
produced in bacteria or yeast cells. However the presently
preferred expression system for biologically active recom-
binant human bone inductive factor is stably transformed CHO
cells.

As one specific example, to produce the human bone
inductive factor (hBMP-1) of Example V, the insert of U2OS-1
is released from the vector arms by digestion with Sal I and
subcloned into the mammalian expression vector pMT2CX digested
with Xho I. Plasmid DNA from this subclone is transfected
into COS cells by the DEAE-dextran procedure [Sompayrac and

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Danna _PNAS_ _78_:7575-7578 (1981); Luthman and Magnusson, _Nucl.Acids Res_. 11: 1295-1308 (1983)]. Serum-free 24 hr. conditioned medium is collected from the cells starting 40-70 hr. post-transfection.

The mammalian expression vector pMT2 Cla-Xho (pMT2 CX) is a derivative of p91023 (b) (Wong et al., Science _228_:810-815, 1985) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 Cla-Xho have been described (Kaufman, R.J., 1985, Proc. Natl. Acad. Sci. USA _82_:689-693) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

Plasmid pMT2 Cla-Xho is obtained by EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRI digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form which can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2CX is then constructed by digesting pMT2 with Eco RV and XbaI, treating the digested DNA with Klenow fragment of DNA polymerase I, and ligating Cla linkers (NEBiolabs, CATCGATG). This removes bases 2266 to 2421 starting from the Hind III site near the SV40 origin of replication and enhancer sequences of pMT2. Plasmid DNA is then digested with EcoRI, blunted as above, and ligated to an EcoRI adapter,

$$5' \ PO_4\text{-AATTCCTCGAGAGCT} \ 3'$$

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3' GGAGCTCTCGA 5'

digested with XhoI, and ligated, yielding pMT2 Cla-Xho, which may then be used to transform E. coli to ampicillin resistance. Plasmid pMT2 Cla-Xho DNA may be prepared by conventional methods.

Example VII

Biological Activity of Expressed Bone Inductive Factor

A. BMP-1

To measure the biological activity of the expressed bone inductive factor (hBMP-1) obtained in Example VI above. The factor is partially purified on a Heparin Sepharose column. 4 ml of transfection supernatant from one 100 mm dish is concentrated approximately 10 fold by ultrafiltration on a YM 10 membrane and then dialyzed against 20mM Tris, 0.15 M NaCl, pH 7.4 (starting buffer). This material is then applied to a 1.1 ml Heparin Sepharose column in starting buffer. Unbound proteins are removed by an 8 ml wash of starting buffer, and bound proteins, including BMP-1, are desorbed by a 3-4 ml wash of 20 mM Tris, 2.0 M NaCl, pH 7.4.

The proteins bound by the Heparin column are concentrated approximately 10-fold on a Centricon 10 and the salt reduced by diafiltration with 0.1% trifluoroacetic acid. The appropriate amount of this solution is mixed with 20 mg of rat matrix and then assayed for in vivo bone and cartilage formation as previously described in Example III. A mock transfection supernatant fractionation is used as a control.

The implants containing rat matrix to which specific amounts of human BMP-1 have been added are removed from rats after seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated.

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Addition of human BMP-1 to the matrix material resulted in formation of cartilage-like nodules at 7 days post implantation. The chondroblast-type cells were recognizable by shape and expression of metachromatic matrix. The amount of activity observed for human BMP-1 was dependent upon the amount of human BMP-1 protein added to the matrix. Table IX illustrates the dose-response relationship of human BMP-1 protein to the amount of bone induction observed.

<u>Table IX</u>

<u>IMPLANT NUMBER</u>	<u>AMOUNT USED</u> (equivalent of ml transfection media)	<u>HISTOLOGICAL SCORE</u>
876-134-1	10 BMP-1	C+2
876-134-2	3 BMP-1	C+1
876-134-3	1 BMP-1	C +/-
876-134-4	10 MOCK	C -
876-134-5	3 MOCK	C -
876-134-6	1 MOCK	C -

Cartilage (c) activity was scored on a scale from 0(-) to 5.

Similar levels of activity are seen in the Heparin Sepharose fractionated COS cell extracts. Partial purification is accomplished in a similar manner as described above except that 6 M urea is included in all the buffers. Further, in a rat bone formation assay as described above, BMP-2 has similarly demonstrated chondrogenic activity.
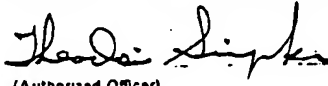
The procedures described above may be employed to isolate other bone inductive factors of interest by utilizing the bovine bone inductive factors and/or human bone inductive factors as a probe source. Such other bone inductive factors may find similar utility in, inter alia, fracture repair.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications

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and variations in practice thereof are expected to occur to
those skilled in the art upon consideration of these descrip-
tions.   Those modifications and variations are believed to be
encompassed within the claims appended hereto.

International Application No: PCT/   /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page _____, line _____ of the description ¹

A. IDENTIFICATION OF DEPOSIT ²

Further deposits are identified on an additional sheet ☐ ³

Name of depositary institution ⁴

American Type Culture Collection

Address of depositary institution (including postal code and country) ⁴

12301 Parklawn Drive
Rockville, Maryland 20852   USA

Name of Deposit	ATCC No.	Referred to on page/line	Date of Deposit
LP-H1	40311	29/20	March 4, 1987
bP50	40295	20/3	December 15, 1986
bP-21	40310	22/18	March 4, 1987
U2OS-3	40342	44/22	June 16, 1987
Lambda U2-OS-1	40343	32/33	June 16, 1987
Lambda BP819	40344	25/23	June 16, 1987
U2OS-39	40345	39/21	June 16, 1987

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ³ (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS ⁸ (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later ⁹ (Specify the general nature of the indications e.g., " Accession Number of Deposit ")

E. ☑ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau ¹⁰

(Authorized Officer)

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WHAT IS CLAIMED IS:

1.. A pharmaceutical composition comprising a protein selected from the group consisting of:

    (a) BMP-1;

    (b) BMP-2 Class I;

    (c) BMP-2 Class II;

    (d) BMP-3; and

mixtures thereof, in a pharmaceutically acceptable vehicle.

2.. A composition of Claim 1 wherein said protein is BMP-1..

3.. A composition of Claim 1 wherein said protein is BMP-2 Class I.

4. A composition of Claim 1 wherein said protein is BMP-2 Class II.

5. A composition of Claim 1 wherein said protein is BMP-3.

6. The pharmaceutical composition of Claim 1 further comprising a matrix capable of delivering the composition to the site of the bone defect and providing a structure for inducing bone formation.

7.. The composition of Claim 6 wherein said matrix comprises a material selected from the group consisting of hydroxyapatite, collagen, polylactic acid and tricalcium phosphate.

8.. A method for inducing bone formation in a patient in need of same comprising administering to said patient an effective amount of a composition of Claim 1-7.

9. A process for producing BMP-1 comprising culturing in a suitable culture medium a cell line transformed with a DNA sequence encoding BMP-1, said DNA sequence being in relative association with an expression control sequence therefor, and isolating BMP-1 from said culture medium.

10. A process according to Claim 9 wherein said DNA sequence comprises substantially the nucleotide sequence of Table VI.

11. A process for producing BMP-2 Class I comprising culturing in a suitable culture medium a cell line transformed

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with a DNA sequence encoding BMP-2 Class I, said DNA sequence being in relative association with an expression control sequence therefor, and isolating BMP-2 Class I from said culture medium.

12.   A process for according to Claim 11 wherein said DNA sequence comprises substantially the nucleotide sequence of Table VII.

13..   A process for producing BMP-2 Class II comprising culturing in a suitable culture medium a cell line transformed with a DNA sequence encoding BMP-2 Class II, said DNA sequence being in relative association with an expression control sequence therefor, and isolating BMP-2 Class II from said culture medium.

14.   A process according to Claim 13 wherein said DNA sequence comprises substantially the nucleotide sequence of Table VIII.

15.   A process for producing BMP-3 comprising culturing in a suitable culture medium a cell line transformed with a DNA sequence encoding BMP-3, said DNA sequence being in relative association with an expression control sequence therefor and isolating BMP-3 from said culture medium.

16.   A process according to Claim 15 wherein said DNA sequence comprises substantially the nucleotide sequence of Table IVA and IVB.

17.   A cDNA sequence encoding BMP-1 comprising substantially the nucleotide sequence of Table VI or a sequence which hybridize thereto under stringent conditions and which upon expression codes for a protein exhibiting substantial properties of BMP-1.

18.   A cDNA sequence encoding BMP-2 Class I comprising substantially the nucleotide sequence of Table VII or a sequence which hybridizes thereto under stringent conditions and which upon expression codes for a protein exhibiting substantial properties of BMP-2 Class I.

19.   A cDNA sequence encoding BMP-2 Class II comprising

substantially the nucleotide sequence of Table VIII or a
sequence which hybridizes thereto under stringent conditions
and which upon expression codes for a protein exhibiting
substantial properties of BMP-2 Class II.

20.   A cDNA sequence encoding BMP-3 comprising substantially
the nucleotide sequence of Table IVA and IVB or a sequence
which hybridizes thereto under stringent conditions and which
upon expression codes for a protein exhibiting substantial
properties of BMP-3.

AMENDED CLAIMS
[received by the International Bureau on 8 December 1987 (08.12.87)
original claims 6, 8, 10, 12, 14, 16-20 amended;
new claims 21-23 added; other claims unchanged (13 pages)]

1.   A pharmaceutical composition comprising a protein selected from the group consisting of:

       (a)   BMP-1;

       (b)   BMP-2 Class I;

       (c)   BMP-2 Class II;

       (d)   BMP-3; and

mixtures thereof, in a pharmaceutically acceptable vehicle.

2.   A composition of Claim 1 wherein said protein is BMP-1.

3.   A composition of Claim 1 wherein said protein is BMP-2 Class I.

4.   A composition of Claim 1 wherein said protein is BMP-2 Class II.

5.   A composition of Claim 1 wherein said protein is BMP-3.

6.   The pharmaceutical composition of Claim 1 further comprising a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.

7.   The composition of Claim 6 wherein said matrix comprises a material selected from the group consisting of hydroxyapatite, collagen, polylactic acid and tricalcium phosphate.

8.   A method for inducing bone or cartilage formation in a patient in need of same comprising administering to said patient an effective amount of a composition of Claim 1-7.

9.   A process for producing BMP-1 comprising the steps of
culturing in a suitable culture medium a host cell
transformed with a DNA sequence encoding BMP-1, said DNA
sequence being in relative association with an expression
control sequence therefor; and isolating said BMP-1
therefrom.

10. A process according to Claim 9 wherein said DNA sequence
comprises substantially the nucleotide sequence as follows:

```
          10        20        30                   50
CTAGAGGCCG CTTCCCTCGC CGCCGCCCCG CCAGC ATG CCC GGC GTG GCC CGC CTG CCG
                                        MET Pro Gly Val Ala Arg Leu Pro


       65                  80                  95                 110
CTG CTG CTC GGG CTG CTG CTG CTC CCG CGT CCC GGC CGG CCG CTG GAC TTG GCC
Leu Leu Leu Gly Leu Leu Leu Leu Pro Arg Pro Gly Arg Pro Leu Asp Leu Ala


             125                 140                 155
GAC TAC ACC TAT GAC CTG GCG GAG GAG GAC GAC TCG GAG CCC CTC AAC TAC AAA
Asp Tyr Thr Tyr Asp Leu Ala Glu Glu Asp Asp Ser Glu Pro Leu Asn Tyr Lys


170                 185                 200                 215
GAC CCC TGC AAG GCG GCT GCC TTT CTT GGG GAC ATT GCC CTG GAC GAA GAG GAC
Asp Pro Cys Lys Ala Ala Ala Phe Leu Gly Asp Ile Ala Leu Asp Glu Glu Asp


             230                 245                 260                 275
CTG AGG GCC TTC CAG GTA CAG CAG GCT GTG GAT CTC AGA CGG CAC ACA GCT CGT
Leu Arg Ala Phe Gln Val Gln Gln Ala Val Asp Leu Arg Arg His Thr Ala Arg


                   290                 305                 320
AAG TCC TCC ATC AAA GCT GCA GTT CCA GGA AAC ACT TCT ACC CCC AGC TGC CAG
Lys Ser Ser Ile Lys Ala Ala Val Pro Gly Asn Thr Ser Thr Pro Ser Cys Gln


335                 350                 365                 380
AGC ACC AAC GGG CAG CCT CAG AGG GGA GCC TGT GGG AGA TGG AGA GGT AGA TCC
Ser Thr Asn Gly Gln Pro Gln Arg Gly Ala Cys Gly Arg Trp Arg Gly Arg Ser


             395                 410                 425
CGT AGC CGG CGG GCG GCG ACG TCC CGA CCA GAG CGT GTG TGG CCC GAT GGG GTC
Arg Ser Arg Arg Ala Ala Thr Ser Arg Pro Glu Arg Val Trp Pro Asp Gly Val


440                 455                 470                 485
ATC CCC TTT GTC ATT GGG GGA AAC TTC ACT GGT AGC CAG AGG GCA GTC TTC CGG
Ile Pro Phe Val Ile Gly Gly Asn Phe Thr Gly Ser Gln Arg Ala Val Phe Arg


             500                 515                 530                 545
CAG GCC ATG AGG CAC TGG GAG AAG CAC ACC TGT GTC ACC TTC CTG GAG CGC ACT
Gln Ala MET Arg His Trp Glu Lys His Thr Cys Val Thr Phe Leu Glu Arg Thr
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          560                      575                      590
GAC GAG GAC AGC TAT ATT GTG TTC ACC TAT CGA CCT TGC GGG TGC TGC TCC TAC
Asp Glu Asp Ser Tyr Ile Val Phe Thr Tyr Arg Pro Cys Gly Cys Cys Ser Tyr

          605                      620                      635          650
GTG GGT CGC CGC GGC GGG GGC CCC CAG GCC ATC TCC ATC GGC AAG AAC TGT GAC
Val Gly Arg Arg Gly Gly Gly Pro Gln Ala Ile Ser Ile Gly Lys Asn Cys Asp

          665                      680                      695
AAG TTC GGC ATT GTG GTC CAC GAG CTG GGC CAC GTC GTC GGC TTC TGG CAC GAA
Lys Phe Gly Ile Val Val His Glu Leu Gly His Val Val Gly Phe Trp His Glu

710                      725                      740                      755
CAC ACT CGG CCA GAC CGG GAC CGC CAC GTT TCC ATC GTT CGT GAG AAC ATC CAG
His Thr Arg Pro Asp Arg Asp Arg His Val Ser Ile Val Arg Glu Asn Ile Gln

          770                      785                      800          815
CCA GGG CAG GAG TAT AAC TTC CTG AAG ATG GAG CCT CAG GAG GTG GAG TCC CTG
Pro Gly Gln Glu Tyr Asn Phe Leu Lys MET Glu Pro Gln Glu Val Glu Ser Leu

                    830                      845                      860
GGG GAG ACC TAT GAC TTC GAC AGC ATC ATG CAT TAC GCT CGG AAC ACA TTC TCC
Gly Glu Thr Tyr Asp Phe Asp Ser Ile MET His Tyr Ala Arg Asn Thr Phe Ser

          875                      890                      905          920
AGG GGC ATC TTC CTG GAT ACC ATT GTC CCC AAG TAT GAG GTG AAC GGG GTG AAA
Arg Gly Ile Phe Leu Asp Thr Ile Val Pro Lys Tyr Glu Val Asn Gly Val Lys

                    935                      950                      965
CCT CCC ATT GGC CAA AGG ACA CGG CTC AGC AAG GGG GAC ATT GCC CAA GCC CGC
Pro Pro Ile Gly Gln Arg Thr Arg Leu Ser Lys Gly Asp Ile Ala Gln Ala Arg

980                      995                   1010                      1025
AAG CTT TAC AAG TGC CCA GCC TGT GGA GAG ACC CTG CAA GAC AGC ACA GGC AAC
Lys Leu Tyr Lys Cys Pro Ala Cys Gly Glu Thr Leu Gln Asp Ser Thr Gly Asn

          1040                     1055                     1070          1085
TTC TCC TCC CCT GAA TAC CCC AAT GGC TAC TCT GCT CAC ATG CAC TGC GTG TGG
Phe Ser Ser Pro Glu Tyr Pro Asn Gly Tyr Ser Ala His MET His Cys Val Trp

                    1100                     1115                     1130
CGC ATC TCT GTC ACA CCC GGG GAG AAG ATC ATC CTG AAC TTC ACG TCC CTG GAC
Arg Ile Ser Val Thr Pro Gly Glu Lys Ile Ile Leu Asn Phe Thr Ser Leu Asp

          1145                     1160                     1175          1190
CTG TAC CGC AGC CGC CTG TGC TGG TAC GAC TAT GTG GAG GTC CGA GAT GGC TTC
Leu Tyr Arg Ser Arg Leu Cys Trp Tyr Asp Tyr Val Glu Val Arg Asp Gly Phe

          1205                     1220                     1235
TGG AGG AAG GCG CCC CTC CGA GGC CGC TTC TGC GGG TCC AAA CTC CCT GAG CCT
Trp Arg Lys Ala Pro Leu Arg Gly Arg Phe Cys Gly Ser Lys Leu Pro Glu Pro
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     1250                1265                1280                1295
ATC GTC TCC ACT GAC AGC CGC CTC TGG GTT GAA TTC CGC AGC AGC AGC AAT TGG
Ile Val Ser Thr Asp Ser Arg Leu Trp Val Glu Phe Arg Ser Ser Ser Asn Trp


     1310                1325                1340                1355
GTT GGA AAG GGC TTC TTT GCA GTC TAC GAA GCC ATC TGC GGG GGT GAT GTG AAA
Val Gly Lys Gly Phe Phe Ala Val Tyr Glu Ala Ile Cys Gly Gly Asp Val Lys


          1370                1385                1400
AAG GAC TAT GGC CAC ATT CAA TCG CCC AAC TAC CCA GAC GAT TAC CGG CCC AGC
Lys Asp Tyr Gly His Ile Gln Ser Pro Asn Tyr Pro Asp Asp Tyr Arg Pro Ser


     1415                1430                1445                1460
AAA GTC TGC ATC TGG CGG ATC CAG GTG TCT GAG GGC TTC CAC GTG GGC CTC ACA
Lys Val Cys Ile Trp Arg Ile Gln Val Ser Glu Gly Phe His Val Gly Leu Thr


          1475                1490                1505
TTC CAG TCC TTT GAG ATT GAG CGC CAC GAC AGC TGT GCC TAC GAC TAT CTG GAG
Phe Gln Ser Phe Glu Ile Glu Arg His Asp Ser Cys Ala Tyr Asp Tyr Leu Glu


  1520                1535                1550                1565
GTG CGC GAC GGG CAC AGT GAG AGC AGC ACC CTC ATC GGG CGC TAC TGT GGC TAT
Val Arg Asp Gly His Ser Glu Ser Ser Thr Leu Ile Gly Arg Tyr Cys Gly Tyr


          1580                1595                1610                1625
GAG AAG CCT GAT GAC ATC AAG AGC ACG TCC AGC CGC CTC TGG CTC AAG TTC GTC
Glu Lys Pro Asp Asp Ile Lys Ser Thr Ser Ser Arg Leu Trp Leu Lys Phe Val


          1640                1655                1670
TCT GAC GGG TCC ATT AAC AAA GCG GGC TTT GCC GTC AAC TTT TTC AAA GAG GTG
Ser Asp Gly Ser Ile Asn Lys Ala Gly Phe Ala Val Asn Phe Phe Lys Glu Val


     1685                1700                1715                1730
GAC GAG TGC TCT CGG CCC AAC CGC GGG GGC TGT GAG CAG CGG TGC CTC AAC ACC
Asp Glu Cys Ser Arg Pro Asn Arg Gly Gly Cys Glu Gln Arg Cys Leu Asn Thr


          1745                1760                1775
CTG GGC AGC TAC AAG TGC AGC TGT GAC CCC GGG TAC GAG CTG GCC CCA GAC AAG
Leu Gly Ser Tyr Lys Cys Ser Cys Asp Pro Gly Tyr Glu Leu Ala Pro Asp Lys


  1790                1805                1820                1835
CGC CGC TGT GAG GCT GCT TGT GGC GGA TTC CTC ACC AAG CTC AAC GGC TCC ATC
Arg Arg Cys Glu Ala Ala Cys Gly Gly Phe Leu Thr Lys Leu Asn Gly Ser Ile


     1850                1865                1880                1895
ACC AGC CCG GGC TGG CCC AAG GAG TAC CCC CCC AAC AAG AAC TGC ATC TGG CAG
Thr Ser Pro Gly Trp Pro Lys Glu Tyr Pro Pro Asn Lys Asn Cys Ile Trp Gln


          1910                1925                1940
CTG GTG GCC CCC ACC CAG TAC CGC ATC TCC CTG CAG TTT GAC TTC TTT GAG ACA
Leu Val Ala Pro Thr Gln Tyr Arg Ile Ser Leu Gln Phe Asp Phe Phe Glu Thr
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          1955            1970              1985              2000
GAG GGC AAT GAT GTG TGC AAG TAC GAC TTC GTG GAG GTG CGC AGT GGA CTC ACA
Glu Gly Asn Asp Val Cys Lys Tyr Asp Phe Val Glu Val Arg Ser Gly Leu Thr

          2015              2030              2045
GCT GAC TCC AAG CTG CAT GGC AAG TTC TGT GGT TCT GAG AAG CCC GAG GTC ATC
Ala Asp Ser Lys Leu His Gly Lys Phe Cys Gly Ser Glu Lys Pro Glu Val Ile

    2060            2075              2090              2105
ACC TCC CAG TAC AAC AAC ATG CGC GTG GAG TTC AAG TCC GAC AAC ACC GTG TCC
Thr Ser Gln Tyr Asn Asn MET Arg Val Glu Phe Lys Ser Asp Asn Thr Val Ser

          2120              2135              2150              2165
AAA AAG GGC TTC AAG GCC CAC TTC TTC TCA GAA AAG AGG CCA GCT CTG CAG CCC
Lys Lys Gly Phe Lys Ala His Phe Phe Ser Glu Lys Arg Pro Ala Leu Gln Pro

          2180              2195              2210
CCT CGG GGA CGC CCC CAC CAG CTC AAA TTC CGA GTG CAG AAA AGA AAC CGG ACC
Pro Arg Gly Arg Pro His Gln Leu Lys Phe Arg Val Gln Lys Arg Asn Arg Thr

    2225      2235      2245      2255      2265      2275      2285
CCC CAG TGAGGCCTGC CAGGCCTCCC GGACCCCTTG TTACTCAGGA ACCTCACCTT GGACGGAATG
Pro Gln

    2295      2305      2315      2325      2335      2345      2355
GGATGGGGGC TTCGGTGCCC ACCAACCCCC CACCTCCACT CTGCCATTCC GGCCCACCTC CCTCTGGCCG

    2365      2375      2385      2395      2405      2415      2425
GACAGAACTG GTGCTCTCTT CTCCCCACTG TGCCCGTCCG CGGACCGGGG ACCCTTCCCC GTGCCCTACC

    2435      2445      2455      2465      2475      2485      2495
CCCTCCCATT TTGATGGTGT CTGTGACATT TCCTGTTGTG AAGTAAAAGA GGGACCCCTG CGTCCTGCCT
```

CTAGA

11. A process for producing BMP-2 Class I comprising culturing
in a suitable culture medium a cell line transformed with a DNA
sequence encoding BMP-2 Class I, said DNA sequence being in
relative association with an expression control sequence
therefor, and isolating BMP-2 Class I from said culture medium.

12. A process for according to Claim 11 wherein said DNA
sequence comprises substantially the nucleotide sequence as

follows:

```
        10         20         30         40         50         60         70
GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT AACTTGCGCA

        80         90        100        110        120        130        140
CCCCACTTTG CGCCGGTGCC TTTGCCCCAG CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCTCC

       150        160        170        180        190        200        210
ACTCCTCGGC CTTGCCCGAC ACTGAGACGC TGTTCCCAGC GTGAAAAGAG AGACTGCGCG GCCGGCACCC

       220        230        240        250        260        270        280
GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTCGGT CCTTGCGCCA GGTCCTTTGA CCAGAGTTTT

       290        300        310        320        330        340        350
TCCATGTGGA CGCTCTTTCA ATGGACGTGT CCCCGCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT
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                              370                   385                   400
CGACC ATG GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC
      MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val

                        415                   430                   445
CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG
Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala

460                   475                   490                   505
GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG
Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu

        520                   535                   550                   565
TTC GAG TTG CGG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC
Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser

                  580                   595                   610
AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCG GGT
Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly

        625                   640                   655                   670
CAG CCG GGC TCA CCC GCC CCA GAC CAC CGG TTG GAG AGG GCA GCC AGC CGA GCC
Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala

                  685                   700                   715
AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG
Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

730                   745                   760                   775
AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu

        790                   805                   820                   835
GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala
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                850                      865                       880
TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys


            895                      910                       925                       940
CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu


                955                      970                       985
GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET


  1000                1015                      1030                       1045
CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC
Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His


                1060                      1075                      1090                       1105
TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu


                1120                      1135                      1150
CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly


    1165                      1180                      1195                      1210
CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC
His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His


                1225                      1240                      1255
AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC
Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp


  1270                1285                      1300                       1315
TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC
Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala


                1330                      1345                      1360                       1375
TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT
Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr


                1390                      1405                      1420
AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG
Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys


    1435                      1450                      1465                      1480
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
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        1495              1510              1525
AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

1540      1553      1563      1573      1583      1593      1603
TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
Cys Arg

AAAA
```

13. A process for producing BMP-2 Class II comprising culturing
in a suitable culture medium a cell line transformed with a DNA
sequence encoding BMP-2 Class II, said DNA sequence being in
relative association with an expression control sequence
therefor, and isolating BMP-2 Class II from said culture medium.

14. A process according to Claim 13 wherein said DNA sequence
comprises substantially the nucleotide sequence as follows:

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        10        20        30        40        50        60        70
CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA GGTGAGTGTG

        80        90        100       110       120       130       140
GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC

        150       160       170       180       190       200       210
GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC AGGTTCACTG

        220       230       240       250       260       270       280
CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC

        290       300       310       320       330       340       350
GTAGTGCCAT CCCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG

        360       370       380       390       400
CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT
                                                          MET Ile Pro

        417              432               447              462
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala

        477              492              507
AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln
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522                 537                 552                 567
GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe

        582                 597                 612                 627
GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys

                642                 657                 672
AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

        687                 702                 717                 732
GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC
Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

            747                 762                 777
AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile

792                 807                 822                 837
CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

        852                 867                 882                 897
CCT GAG AAC GAG GCG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG
Pro Glu Asn Glu Ala Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

            912                 927                 942
GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

957                 972                 987                 1002
ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

        1017                1032                1047
ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT
Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

1062                1077                1092                1107
GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG
Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

        1122                1137                1152                1167
GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

            1182                1197                1212
CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC
Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
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          1227                1242                   1257                1272
    ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG
    Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys


              1287                1302                1317
    CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
    Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg


    1332                1347                   1362                1377
    CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
    Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val


          1392                1407                1422                   1437
    GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
    Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu


              1452                1467                1482
    GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
    Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser


       1497                1512                   1527                1542
    GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
    Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile


              1557                1572                1587
    TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
    Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu


    1602           1617                   1636      1646       1656       1666
    ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG ATATACACAC
    MET Val Val Glu Gly Cys Gly Cys Arg


         1676       1686       1696       1706       1716       1726       1736
    CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC ACAGACTGCT


         1746       1756       1766       1776       1786       1796       1806
    TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC ATTCACCTTG


         1816       1826       1836       1846       1856       1866       1876
    ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA ATATATTTAT


         1886       1896       1906       1916       1926       1936       1946
    AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAAACT CTAGAGTCGA

    CGGAATTC
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15. A process for producing BMP-3 comprising culturing in a
suitable culture medium a cell line transformed with a DNA
sequence encoding BMP-3, said DNA sequence being in relative

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association with an expression control sequence therefor and isolating BMP-3 from said culture medium.

16. A process according to Claim 15 wherein said DNA sequence comprises substantially the nucleotide sequence as follows:

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       383        393        403        413                   428
GAGGAGGAAG CGGTCTACGG GGGTCCTTCT GCCTCTGCAG   AAC AAT GAG CTT CCT GGG GCA
                                              Asn Asn Glu Leu Pro Gly Ala


           443                    458              473                488
GAA TAT CAG TAC AAG GAG GAT GAA GTA TGG GAG GAG AGG AAG CCT TAC AAG ACT
Glu Tyr Gln Tyr Lys Glu Asp Glu Val Trp Glu Glu Arg Lys Pro Tyr Lys Thr


                   503              518              533
CTT CAG ACT CAG CCC CCT GAT AAG AGT AAG AAC AAA AAG AAA CAG AGG AAG GGA
Leu Gln Thr Gln Pro Pro Asp Lys Ser Lys Asn Lys Lys Lys Gln Arg Lys Gly


       548              563              578              593
CCT CAG CAG AAG AGT CAG ACG CTC CAG TTT GAT GAA CAG ACC CTG AAG AAG GCA
Pro Gln Gln Lys Ser Gln Thr Leu Gln Phe Asp Glu Gln Thr Leu Lys Lys Ala


           608              623              638
AGA AGA AAG CAA TGG ATT GAA CCC CGG AAT TGT GCC AGA CGG TAC CTT AAA GTG
Arg Arg Lys Gln Trp Ile Glu Pro Arg Asn Cys Ala Arg Arg Tyr Leu Lys Val


653              668              683              698
GAC TTC GCA GAT ATT GGC TGG AGC GAA TGG ATT ATT TCC CCC AAG TCC TTC GAT
Asp Phe Ala Asp Ile Gly Trp Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp


       713              728              743         756         766
GCC TAT TAC TGC TCC GGA GCG TGC CAG TTC CCC ATG CCA AAG GTAGCCATTG TTTTTTGTCC
Ala Tyr Tyr Cys Ser Gly Ala Cys Gln Phe Pro MET Pro Lys


       776        786
TGTCCTTCCC ATTTCCATAG ; and


       284        294        304                   319
CTAACCTGTG TTCTCCCTTT TCGTTCCTAG   TCT TTG AAG CCA TCA AAT CAC GCT ACC
                                   Ser Leu Lys Pro Ser Asn His Ala Thr


334              349              364              379
ATC CAG AGT ATA GTG AGA GCT GTG GGG GTC GTC CCT GGA ATC CCC GAG CCT TGC
Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile Pro Glu Pro Cys


       394              409     ,        424              439
TGT GTG CCA GAA AAG ATG TCC TCA CTC AGC ATC TTA TTC TTT GAT GAA AAC AAG
Cys Val Pro Glu Lys MET Ser Ser Leu Ser Ile Leu Phe Phe Asp Glu Asn Lys
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        454              469              484
AAT GTG GTA CTT AAA GTA TAT CCA AAC ATG ACA GTA GAG TCT TGT GCT TGC AGA
Asn Val Val Leu Lys Val Tyr Pro Asn MET Thr Val Glu Ser Cys Ala Cys Arg

    503     513     523     533
TAACCTGGTG AAGAACTCAT CTGGATGCTT AACTCAATCG.
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17. A cDNA sequence encoding BMP-1 comprising substantially the nucleotide sequence recited in Claim 10 or a sequence which hybridizes thereto under stringent conditions and which upon expression codes for a protein exhibiting substantial properties of BMP-1.

18. A cDNA sequence encoding BMP-2 Class I comprising substantially the nucleotide sequence recited in Claim 12 or a sequence which hybridizes thereto under stringent conditions and which upon expression codes for a protein exhibiting substantial properties of BMP-2 Class I.

19. A cDNA sequence encoding BMP-2 Class II comprising substantially the nucleotide sequence recited in Claim 14 or a sequence which hybridizes thereto under stringent conditions and which upon expression codes for a protein exhibiting substantial properties of BMP-2 Class II.

20. A cDNA sequence encoding BMP-3 comprising substantially the nucleotide sequence recited in Claim 16 or a sequence which hybridizes thereto under stringent conditions and which upon expression codes for a protein exhibiting substantial properties of BMP-3.

21. A vector containing a DNA sequence encoding an osteoinductive protein and heterologous DNA, the DNA sequence encoding the protein being selected from the group consisting of:
    a. a DNA sequence encoding BMP-1 comprising substantially the nucleotide sequence recited in Claim 10 or a sequence which

hybridize thereto under stringent conditions and which upon
expression codes for a protein exhibiting substantial properties
of BMP-1;

   b. a DNA sequence encoding BMP-2 Class I comprising
substantially the nucleotide sequence recited in Claim 12 or a
sequence which hybridizes thereto under stringent conditions and
which upon expression codes for a protein exhibiting substantial
properties of BMP-2 Class I;

   c. a DNA sequence encoding BMP-2 Class II comprising
substantially the nucleotide sequence recited in Claim 14 or a
sequence which hybridizes thereto under stringent conditions and
which upon expression codes for a protein exhibiting substantial
properties of BMP-2 Class II; and

   d. a DNA sequence encoding BMP-3 comprising substantially
the nucleotide sequence recited in Claim 16 or a sequence which
hybridizes thereto under stringent conditions and which upon
expression codes for a protein exhibiting substantial properties
of BMP-3.

22. A cell transformed with a vector according to claim 21 which
is capable of expressing a DNA sequence encoding the
osteoinductive protein and progeny of said cell.

23. The transformed cell according to claim 24 selected from the
group consisting of a mammalian cell, a bacterial cell, an insect
cell, and a yeast cell.

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C07K 13/00,15/00; A61K 37/00; See Attachment
US CL: 530/350,395,397; 514/12; 536/27 See Attachment

II. FIELDS SEARCHED

Minimum Documentation Searched 4	
Classification System	Classification Symbols
US	530/350,395,397; 514/12; 536/27 435/68, 70, 172.3; 935/13

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 6

COMPUTER SEARCH CAS, APS: BONE MORPHOGEN, BONE
INDUCTIVE PROTEIN, BMP, OSTEOINDUCTIVE FACTOR

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X,P Y,P	US, A, 4,619,989 (URIST) 28 Oct 1986.	1-8 9-20
X Y	US, A, 4,563,350 (NATHAN ET AL) 7 January 1986.	1-8 9-20
X Y	US, A, 4,455,256 (URIST) 19 June 1984.	1-8 9-20
X	Proc. Natl. Acad. Sci USA, Vol. 81, issued January 1984, (Washington, D.C.),	1
Y	(URIST), "Purification of bovine morphogenetic protein by hydroxyapatite chromatography", pages 371-375.	2-20

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
08 October 1987	2 0 OCT 1987
International Searching Authority 1	Signature of Authorized Officer 20
ISA/US	Alvin E. Tanenholtz

PCT/US87/01537

Attachment To Form PCT/ISA/210, Part I.

IPC.(4): C12P 21/00, 21/02; C12N 15/00; C07H 15/12
US CL : 435/68, 70, 172.3; 935/13

III. D CUMENTS CONSIDERED TO BE RELEVANT   (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
Y	Science, Vol. 220 issued 13 May 1983 (Washington, D.C.) (URIST) "Bone cell Differentiation and Growth Factors" pages 680-686.	1-20
Y	Proc. Natl. Acad Sci, USA, Vol. 80 issued November 1983 (Washington, D.C.) (SAMPATH ET AL), "Homology of bone-inductive proteins from human monkey, bovine and rat extracellular matrix," pages 6591-6595.	1-20
Y	Proc. Natl. Acad. Sci, USA, Vol. 78 issued November 1981, (Washington, D.C.) (SUGGS ET AL), "Use of synthetic oligonucleotides as hybridization probes: Isolation of cloned cDNA sequence for human β_2-microglobulin" pages 6613-6617.	1-20